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Acute and chronic toxicity of hydrogen sulfide to seven fish species and eight invertebrates were determined in continuous-flow bioassays. Fish species were fathead minnows, goldfish, bluegill, walleye, white sucker, brook trout, and rainbow trout. Invertebrates were *Asellus*, *Crangonyx*, *Gammarus*, *Baetis*, *Hexagenia*, *Ephemera*, *Procambarus*, and *Cambarus*. In 159 acute tests lethal threshold concentration for juvenile fish varied from 0.0087 mg/liter in rainbow trout to 0.0840 mg/liter in goldfish. Except in goldfish, fry stage was up to three times more sensitive than the juvenile. In 96 tests on invertebrates the 96-hr LC50 ranged from 0.020 mg/liter in *Baetis* to 1.070 mg/liter in *Asellus*. Acute toxicity of H_2S to fathead minnows varied 24-fold between 6.5 and 24.0 C. Temperature effects were not as marked on invertebrates. In chronic exposure to H_2S in 29 tests running up to 825 days, maximum no-effect concentration to fish ranged from 0.0004 mg/liter in bluegills to 0.0100 mg/liter in goldfish. No-effect level was determined from growth, survival, reproduction, or swimming performance. In nine chronic tests running up to 138 days, maximum safe levels ranged from 0.0012 mg/liter in *Gammarus* to 0.0152 mg/liter in *Hexagenia*. Application factors relating acute toxic (96-hr LC50 for juveniles) to no-effect levels varied from .231 in rainbow trout to .013 in bluegills and from .091 in *Gammarus* to .048 in *Procambarus*.

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EFFECT OF HYDROGEN SULFIDE ON FISH AND INVERTEBRATES

Part I - Acute and Chronic Toxicity Studies

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ABSTRACT

Acute and chronic toxicity of hydrogen sulfide to seven fish species and eight invertebrates were determined in continuous-flow bioassays. Fish species were fathead minnows, goldfish, bluegill, walleye, white sucker, brook trout, and rainbow trout. Invertebrates were Asellus, Crangonyx, Gammarus, Baetis, Hexagenia, Ephemera, Procambarus, and Cambarus. In 159 acute tests lethal threshold concentration for juvenile fish varied from 0.0087 mg/liter in rainbow trout to 0.0840 mg/liter in goldfish. Except in goldfish, fry stage was up to three times more sensitive than the juvenile. In 96 tests on invertebrates the 96-hr LC50 ranged from 0.020 mg/liter in Baetis to 1.070 mg/liter in Asellus. Acute toxicity of H_2S to fathead minnows varied 24-fold between 6.5 and 24.0 C. Temperature effects were not as marked on invertebrates. In chronic exposure to H_2S in 29 tests running up to 825 days, maximum no-effect concentration to fish ranged from 0.0004 mg/liter in bluegills to 0.0100 mg/liter in goldfish. No-effect level was determined from growth, survival, reproduction, or swimming performance. In nine chronic tests running up to 138 days, maximum safe levels ranged from 0.0012 mg/liter in Gammarus to 0.0152 mg/liter in Hexagenia. Application factors relating acute toxic (96-hr LC50 for juveniles) to no-effect levels varied from .231 in rainbow trout to .013 in bluegills and from .091 in Gammarus to .048 in Procambarus.

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SECTION I

CONCLUSIONS

The results described in the following report permit certain basic conclusions to be made concerning the toxicity of dissolved undissociated hydrogen sulfide to aquatic organisms. These general conclusions are:

1) H_2S is highly toxic to fish and other aquatic organisms at concentrations frequently found in natural and polluted situations.
2) Toxic concentrations because of their low level or location in the ecosystem are frequently overlooked and their importance not evaluated.
3) The most sensitive life history stage is not the same in all species.
4) Acute toxicity estimates will vary with fish species, source of fish, and temperature of test.
5) The ratio of the no-effect level of H_2S to the 96-hr LC50 varies from 1/3-5 in trout to 1/15-20 in warmwater fish.
6) Recommended H_2S levels for fish will protect macro-invertebrate species.
7) Since threshold LC50 (LTC) concentrations of the most sensitive life history stage in some species approaches the no-effect level, safe level specifications should be 0.05 of the juvenile (LTC) at 20 C in most warmwater species and 0.10 in trout at 15-18 C.
8) To insure no-effect (as defined) on all stages of all warmwater

aquatic species (except some spawning adults) a level of 0.002 mg/liter H_2S at 20 C should be applied.

9) Where fish mortality is associated with oxygen reduction or water movements, hydrogen sulfide levels should be examined.

10) When reproductive behavior brings fish, eggs, or fry into the proximity of areas in which anaerobic decomposition can be expected, such as the soil-water interface, poor reproduction or survival may be caused by continuous evolution of low concentrations of H_2S although dissolved oxygen is adequate.

SECTION II

RECOMMENDATIONS

1. It is recommended that 0.002 mg/liter H_2S be considered a safe limit for protection of all fish species and bottom-inhabiting invertebrates in areas of lakes and streams not used for spawning by nesting fish.
2. It is recommended that in areas used for reproduction by nesting fish that concentrations of 0.001 mg/liter H_2S not be exceeded.
3. It is recommended that appropriate application factors be applied to lethal threshold concentration where particular species are concerned rather than 96-hr LC50.
4. It is recommended that ample oxygen concentration for sustaining fish life not be considered as an index of safe levels of H_2S without analysis for H_2S, particularly in strata of water near the soil-water interface.
5. It is recommended that field studies be conducted to determine the occurrence of H_2S in non-polluted waters to determine natural background levels in potential fish habitats.

SECTION III

INTRODUCTION

A study of the effects of hydrogen sulfide on fish and invertebrates has been conducted over a period of 5 years to determine lethal and sublethal concentrations which have adverse effect on fish and invertebrates. Available literature at the start of the study indicated that there was a wide discrepancy in assumed acute lethal values and that the greater part of the experimental results did not include the conditions under which experimental work had been done. Field work carried out by the principal investigator and his colleagues indicated that very low levels of hydrogen sulfide occurred in nature and in polluted situations and that these levels had detrimental effects on fish reproduction (Colby and Smith[1]; Adelman and Smith[2]). Special gear developed during previous studies enabled the sampling of water near the mud-water interfaces in areas where spawning and egg incubation occurred (Colby and Smith[3]).

Hydrogen sulfide was demonstrated to be constantly evolved from organic sludge in streams and to be a normal product found in hypolimnetic situations during various seasons of the year. In the State of Minnesota hatcheries have been abandoned because of the occurrence of hydrogen sulfide at toxic levels in their water supplies. A variety of industrial effluents contain hydrogen sulfide or their break-down products result in hydrogen sulfide generation. Although hydrogen sulfide oxidizes rapidly, its continued evolution from bottom muds and formation from break-down products may result in a substantial concentration occurring at all times in some fish habitats. The foregoing considerations led to the development of the research program reported herein.

TOXIC PROPERTIES OF HYDROGEN SULFIDE

The toxic properties of hydrogen sulfide are exerted in the undissociated form. The extent of dissociation is strongly dependent on pH with approximately 50% dissociation in the vicinity of 7.0 pH. Hydrogen sulfide affects oxygen relationships in the organism and also may affect mitochondrial systems. The toxicology of hydrogen sulfide in freshwater aquatic organisms has been little studied and there are few definitive references in the literature. A poorly investigated area with reference to effect on organisms has been the actual concentration of undissociated hydrogen sulfide at the point of oxygen transfer on the gills. Discharge of carbon dioxide through gill structures may result in pH levels which alter the apparent hydrogen sulfide level in the ambient solution at the point of bodily entry. Since no direct evaluation of this has been possible, it has been assumed that the ambient levels as determined in the test chambers were the toxic levels. Indirect tests described herein suggest that at high ambient pH, hydrogen sulfide appears more toxic than expected.

BASIS OF INVESTIGATION

The investigations reported here were all conducted in the fishery laboratory of the Department of Entomology, Fisheries, and Wildlife, University of Minnesota, with deep well laboratory water. The organisms used for tests were of wild stock with the exception of laboratory-reared fathead minnows and hatchery-reared goldfish and trout. Both wild and laboratory-reared fathead minnows were used in an extensive series of comparative experiments.

COVERAGE OF PRESENT REPORT

The present report embodies results of chronic and acute tests which established 96-hr LC50 concentrations of hydrogen sulfide and lethal threshold concentration (LTC), defined as the concentration at which no deaths occur for 48 hours, and no-effect levels of the toxicant based on chronic test running up to 826 days. Six species of freshwater fish and eight invertebrates were tested at all life history stages. The problem of application factor to relate acute to chronic responses is discussed in connection with the various species and appropriate factors described for the organisms used.

The seven species of fish used in the investigations were selected to cover a wide spectrum of presumed sensitivity and environmental preference. They included the fathead minnow, *Pimephales promelas* Rafinesque, goldfish, *Carassius auratus* (Linnaeus), white sucker, *Catostomus commersoni* (Lacepede), bluegill, *Lepomis macrochirus* Rafinesque, walleye, *Stizostedion vitreum vitreum* (Mitchill), rainbow trout, *Salmo gairdneri* Richardson, and brook trout, *Salvelinus fontinalis* (Mitchill).

Invertebrates used included two crayfish, *Procambarus clarkii* (Girard) and *Cambarus diogenes* Girard, one isopod, *Asellus militaris* Hay, two amphipods, *Crangonyx richmondensis laurentianus* Bousfield and *Gammarus pseudolimnaeus* Bousfield, and three Ephemeroptera, *Baetis vagans* McDonough, *Ephemera simulans* Walker, and *Hexagenia limbata* (Serville).

OBJECTIVES OF RESEARCH

The specific objectives of the research program were (1) to determine acutely toxic concentrations of hydrogen sulfide (96-hr LC50 and lethal threshold concentration (LTC)); (2) to determine the effect of exposure to chronic low concentrations of hydrogen sulfide on growth, mortality, reproduction, and other responses; (3) to explore factors affecting toxicity of hydrogen sulfide to freshwater organisms; (4) to establish application factors for use with acute tests; and (5) to develop a method for determination of molecular hydrogen sulfide and its first dissociation constants.

PLAN OF PRESENTATION

Presentation of the report has been planned to include most of the pertinent data on materials, methods and results in separate discussions of each species. General aspects of methods applying to all work is included in the Materials and Methods Section. A brief discussion and summary is included at the end of the results section applying to each species. General discussion and comparisons are treated in the section entitled Discussion.

Part I of this report contains all the data on bioassay results, methodology, and evaluation of results and recommendations of safe levels of H_2S for fish and invertebrates. Part II (presented under separate cover) contains discussion of analytical methods for H_2S and a revised set of ionization constants for sulfides dissolved in water.

SECTION IV

MATERIALS AND METHODS

GENERAL LABORATORY CONDITIONS

All water for flow-through tests is transmitted from the well to the laboratory through PVC pipe after catalytic iron removal has reduced Fe to less than 0.1 mg/liter. The water is hard and comes from the Jordan sandstone stratum underlying the Minneapolis-St. Paul metropolitan area (Table 1). Water is cooled when necessary with a Dunham-Bush chiller incorporating stainless steel cooling coils. Heating, aeration, and pH adjustment is done in the head tank above each diluter. Light was controlled automatically to a predetermined schedule according to the needs of individual experiments. Unless otherwise noted, light was from daylight-fluorescent tubes which varied in intensity with various experiments. Ambient room temperature in the laboratory was maintained at 20 C.

TEST ORGANISMS

Fish for experiments were secured from the field or from state and commercial hatcheries. Walleye eggs came from Cutfoot Sioux Lake and fingerlings from various Minnesota State rearing ponds. Fathead minnows came from various lakes and from Duluth-Newtown laboratory stock. Goldfish were all secured from Ozark Fisheries, Inc., Missouri, or were reared in our Laboratory from the same stock. Bluegills came from lakes in the Twin City metropolitan area. White suckers were reared from eggs taken at Bemidji Fishery Station of the Minnesota Department of Natural Resources. Brook trout eggs juveniles, and adults were from the Wis-

Table 1. ANALYSIS OF WELL WATER[a]

(milligrams/liter)

Item	Concentration
Total hardness as $CaCO_3$	220
Calcium as $CaCO_3$	140
Iron	0.02
Chloride	<1
Sulfate	<5
Sulfide	0.0
Fluoride	0.22
Total phosphates	0.03
Sodium	6
Potassium	2
Copper	0.0004
Manganese	0.0287
Zinc	0.0044
Cobalt, nickel	$<$ 0.0005
Cadmium, mercury	$<$ 0.0001
Ammonia nitrogen	0.20
Organic nitrogen	0.20

[a] Water taken from well head before aeration and heating; pH 7.5.

consin State Hatchery at Osceola, Wisconsin. Rainbow trout eggs were purchased from White Trout Farm, Paradise, Utah and from Ennis National Hatchery, Ennis, Montana. Fish were reared in the laboratory. Invertebrates were secured from various sources as described in the discussion of particular experiments.

Prophylactic Treatment

All fish stock was given prophylactic treatment for disease control on entrance to the laboratory. They were subsequently treated in chronic tests when evidence of disease was noted. The protocol for all treatments is described in each species section.

APPARATUS

Acute Tests

Acute test apparatus was of two basic designs, one using H_2S gas and one using sodium sulfide, adjusted pH, and proportional diluters with chemical-metering apparatus ("dipping-bird"-type toxicant dispensers). The gas dispenser is described by Colby and Smith.[1] It consists of two tanks, one containing aerated water and the other oxygen-free water, supplying a constant head of water. Well water prior to entering these tanks is heated in a constant temperature water bath and then aerated with compressed air or stripped of oxygen and carbon dioxide in a second column with nitrogen. Figure 1 is a schematic diagram showing the essential features of the apparatus and the path of the water and H_2S.

The hydrogen sulfide gas passes from a Number 3 cylinder (T) through an H_2S corrosive-free regulator (R), through a needle valve (V), and into the H_2S mixing chamber (X_1). Deoxygenated water (W_1) from one head tank passed through a Roger Gilmont Instruments flowmeter (F) (flow range 10-[illegible] ml/min) and into the mixing chamber (X_1) where the H_2S gas is bubbled through. The mixing chamber (X_1) was made from a sealed [illegible]-liter plastic egg-hatching jar set on a magnetic stirrer (S) with a 1-inch teflon-covered magnet included in the jar. The H_2S content of the water

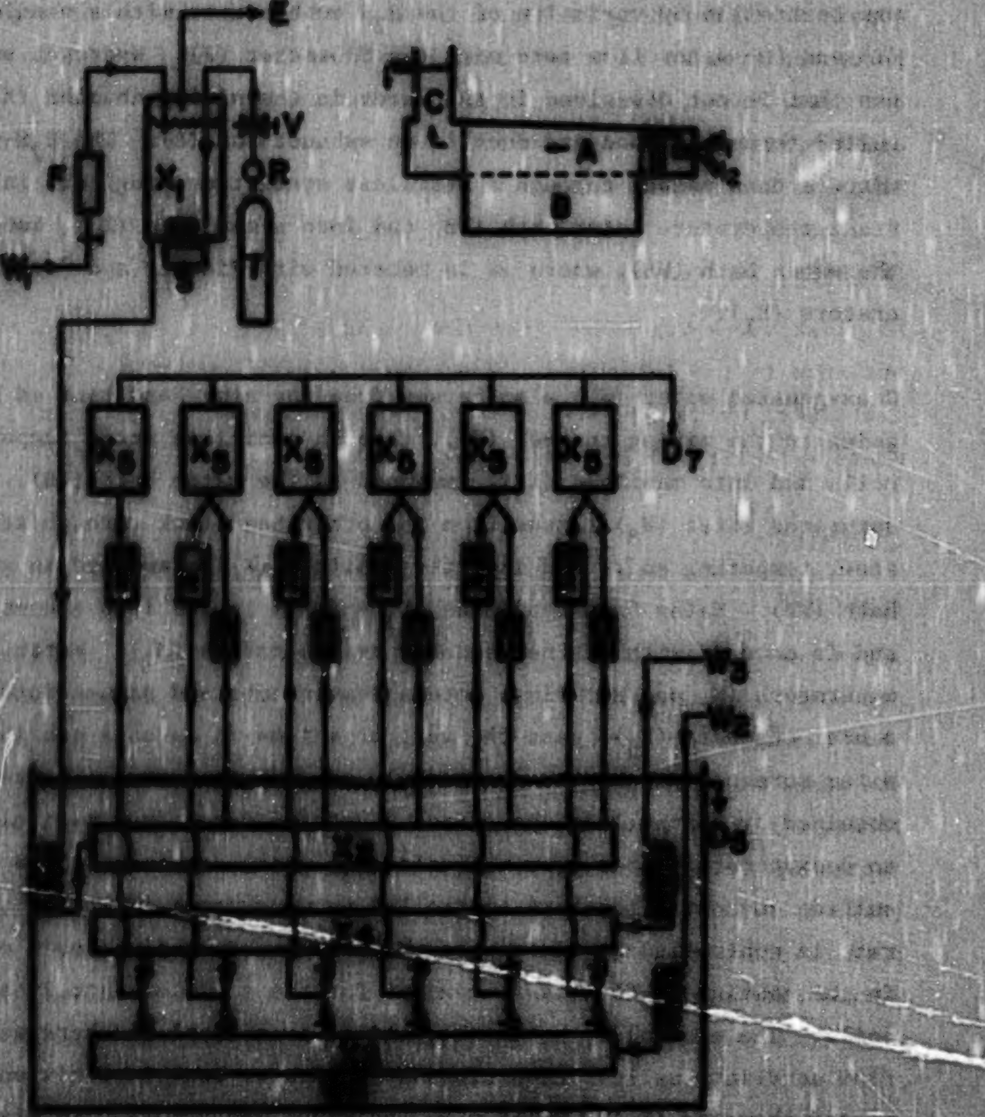

Figure 1. Schematic diagram of the continuous-flow bioassay apparatus showing essential features and path of H_2S and water through the apparatus. H_2S mixing chamber (X_1); manifold for water with dissolved H_2S (X_2); manifold with deoxygenated water (X_3); manifold with aerated water (X_4); environmental chambers (X_5); deoxygenated water (W_1); deoxygenated water (W_2); aerated water (W_3); minimeters (M_1 and M_2); flowmeter (F); H_2S gas tank (T); regulator (R); needle valve (V); exhaust fan (E); magnetic stirrer (S); water bath (WB); water bath drain (D_6); final drain (D_7). Insert of individual environmental chamber (X_5); confinement area of organisms (A); gravel (B); Jarrell-Ash oxygen probe receptacle (C); water with dissolved H_2S (I_1); water adjusted for oxygen concentration (I_2).

was controlled by variation of the H_2S bubble rate with a needle valve (V) and the water flow rate with the flowmeter (F). Hydrogen sulfide gas that is not dissolved in the water in the mixing chamber (X_1) is pulled through a hood and duct to an exhaust fan (E). The H_2S-water mixture then passes through a stainless steel tempering coil in a constant temperature water bath (WB) and into a manifold (X_2), immersed in the water bath (WB), where it is metered with Fischer and Porter Minimeters (M_1).

Deoxygenated water (W_2) also passes from the same head tank as water going to the mixing chamber (X_1), through stainless steel tempering coils and into manifold (X_3), immersed in the water bath (WB). Oxygen-saturated water (W_3) passes from the other head tank through stainless steel tempering coils and into the manifold (X_4), immersed in water bath (WB). Water from manifolds (X_3 and X_4) is fed into a common tube and is metered with Fischer and Porter Minimeters (M_2). Water from minimeters (M_1 and M_2) flows into a common tube and passes into environmental chambers (X_5), past the test organisms in an area confined by nitex screens, and to the drain (X_7). The desired oxygen content is obtained by controlling the ratio of aerated water from manifold (X_4) to deoxygenated water from manifold (X_3) using stopcocks. The desired sulfide concentrations are controlled by minimeters (M_1), and the flow rate is controlled by minimeters (M_2). Tygon tubing was used exclusively in the continuous flow system except for the stainless-steel tempering coils. The manifolds, head tanks and environmental chambers were made from acrylic plastic. Each bioassay apparatus contained six environmental chambers (X_5). Water with the dissolved H_2S (X_1) is mixed with water adjusted for dissolved oxygen concentration (X_2) at a Y junction and enters the chamber through an acrylic plastic tube. Test chambers are designed in accordance with needs of particular organisms as discussed and are given in the discussion of each species.

[illegible] [illegible] [illegible] [illegible] and flow over the test organisms and out of the chamber did not exceed 1-1/2 min. Desired concentrations of dissolved H_2S were attained by adjusting the water flow on the basis of analyzed water from the bioassay test chambers. Flow rates, test chamber size, design and other details peculiar to the tests on each organism are described in the experimental design section relating to each species.

The second type of apparatus used was modified from that described by Brungs and Mount.[4] It was a continuous flow-through type with chemical-metering apparatus (Mount and Brungs[3]) delivering sodium sulfide to each test chamber. Each "dipping bird" in the apparatus was calibrated to deliver the volume required for a particular concentration. Ranges of test concentrations were adjusted by changing concentration of the stock solution. Water flow was controlled by the multiple diluter apparatus. Hydrogen ion concentration was adjusted with H_2SO_4 dispensed with a "dipping bird" apparatus in the head tank above each diluter. Chilled water was introduced to the head tank where needed and final adjustment of temperature made by hot water coils of polyethylene or stainless steel controlled by a thermostat and solenoid in the hot water lines. Flow rates, test chamber size, and variations peculiar to each experiment are described in the design section of the report on that experiment. Desired pH levels were attained by manually adjusting the chemical-metering apparatus on the basis of analysis in the test chambers with a Beckman line-operated meter.

Test chambers in both types of apparatus were of glass or acrylic plastic. Glass chambers were constructed with silicone cement and all connections below the head tank were of glass or tygon tubing.

Chronic Tests

All tests discussed in this report which ran longer than 1 month, including egg to egg tests, are referred to as "chronic tests". Chronic test apparatus was the same as that described above for the sodium sulfide acute tests except that test chambers were varied in size to accommodate the size and numbers of fish used in the particular test. Details of tank size, flow rates, and water retention time are presented in the design section of experiments on each species.

Chemical Analyses

The analytical method used for determination of H_2S was a modification of Method C for sulfide described in Standard Methods for the Examination of Water and Wastewater (American Public Health Association[6]). Samples were taken from the center of the open test chamber and from the outlet in small sealed chambers. They were immediately fixed with zinc acetate; the standard procedures were then followed and absorbance measured with a spectrophotometer. Comparison was made against water from the control test chamber taken at the same time as the sample. Precise repeatability to 0.01 mg/liter total sulfide was attained. At the pH where most bioassays were run (7.7), undissociated H_2S was approximately 0.1 of total sulfide or 0.001 mg/liter with an estimated accuracy of ± 0.0005 mg/liter. Calculation of H_2S was made from Pomeroy[7] tables for pH and temperature. All concentrations of H_2S reported for various tests are from analyses in test chambers and are not nominal or target concentrations based on calculated dilution from stock solutions. Samples were taken three times daily in acute tests and at 1- to 3-day intervals in chronic tests.

After the completion of the bioassay phases of the project, a revised set of ionization constants were experimentally determined. They are discussed in Part II of the present report. A comparison of results determined from the Pomeroy tables with the new determination is made in the discussion section of Part I of this report. Molecular H_2S was determined in laboratory well water and deionized water after addition of a large quantity of sulfide. Results were the same at the same pH and temperature. It is therefore concluded that no materials occur in the well water which interfere with determination of H_2S.

BASIS OF STANDARDS CALCULATION OF LC50 AND LTC

ACUTE TESTS

Median lethal concentrations (LC50) values have been computed by graphic interpolation on semilog paper (American Public Health Association[8]). The median lethal concentrations in most cases were calculated for 48-72 and 96 hours and where possible for lethal threshold concentrations (LTC). Lethal threshold concentration was considered to be reached when no further increase in mortality occurred for 48 hours.

CHRONIC TESTS

The results of chronic tests with levels of H_2S which did not cause death during the first 3 weeks were examined in terms of survival, rate of growth, efficiency of food utilization, success of reproduction, and response to other stresses (including endurance, response to other predators). When a no-effect concentration of H_2S is specified it refers to a level where no demonstrable adverse effect is noted in the parameters measured. In no species were all criteria of adverse effects of sulfide used. Results of criteria used are included with discussion of each species. In some cases (trout) when response was less than 10% [illegible] the controls, the results of treatments were considered to be no different, since the [illegible] between responses of individual fish [illegible] the variability at less than this amount.

[illegible]

[illegible]

All acute tests were done on fish stages not previously subjected to H_2S except as specifically noted. Chronic tests were started with eggs, fry, fingerlings, and adults and results compared. In some species of fish more than one generation was subjected to low concentrations of H_2S. Where possible, chronic tests were run through spawning. Some short term tests for growth rate analysis were also run.

Invertebrate tests were started with eggs, nymphs, or adults in accordance with availability and objective of the test.

Details of design of each experiment are included with the discussion of each particular species.

SECTION V

FATHEAD MINNOW

(*Pimephales promelas* Rafinesque)

The response of the fathead minnow to H_2S was tested by (1) a series of acute bioassays at various temperatures with two stocks of fish, (2) long-term tests at low concentrations, (3) a chronic test through two complete generations, and (4) acute tests on fish from different locations and habitat conditions.

ACUTE BIOASSAY

Experimental Design

Fish for tests described in this section were collected in the St. Paul vicinity (Table 2) and from laboratory stock originating in the National Water Quality Laboratory at Duluth, Minnesota. Fish were treated when brought in and as needed with 20 mg/liter neomycin or tetracycline for 3 days and then with methylene blue at 0.2 mg/liter of 1% solution for each of 3 days. During pretest holding, fish were fed Glencoe dry fish food pellets, Oregon moist pellets, a ground egg and lettuce mixture, and brine shrimp once per day until 24 hr before test. Fish were not fed during first 96 hr of test but daily thereafter. Fish were acclimated to test tanks for 5 days before introduction of toxicant. Eggs and fry were tested in chambers 10 x 16 x 20 cm with three glass sides and bottom and one side of #351 Nitex screen. These test cells were immersed in 30 x 33 x 20 cm glass chambers which were also used for juvenile tests. Eggs rested on the bottom of the small chamber until hatched.

Table 2. SOURCE OF FATHEAD MINNOWS AND STAGE AT START OF ACUTE TESTS WITH H_2S

Test	Stage at start	Stage at collection	Sources	Date of collection	Temperature at collection, C	Method of collection
30	Egg	Egg	Duluth stock	9/12/72	24	Spawning tiles
34	"	"	" "	12/11/72	"	" "
36	"	"	" "	1/15/73	"	" "
40	"	"	" "	2/6/73	"	" "
41	"	"	" "	2/19/73	"	" "
42	"	"	" "	3/4/73	"	" "
43	Fry	"	" "	3/14/73	"	" "
44	"	"	" "	3/21/73	"	" "
45	"	"	" "	3/30/73	"	" "
22	Juvenile	"	" "	11/12/70	20	" "
24	"	"	" "	3/24/71	22	" "
25	"	"	" "	10/18/71	"	" "
26	"	"	" "	10/18/71	"	" "
4	"	Juvenile	Cleveland Pond	10/29/68	9	Seine
7	"	"	" "	10/29/68	"	"
8	"	"	" "	10/29/68	"	"
9	"	"	" "	12/23/68	4	Trap
11	"	"	" "	12/23/68	"	"
12	"	"	" "	8/20/69	22	Seine
13	"	"	Lake Como	3/4/70	4	Trap
16	"	"	" "	7/21/70	23	Seine
17	"	"	" "	9/21/70	19	"
18	"	"	" "	10/12/70	10	"
19	"	"	" "	11/2/70	6	"
20	"	"	" "	1/4/71	4	Trap
21	"	"	" "	1/12/71	"	"

Table 2 (continued). SOURCE OF FATHEAD MINNOWS AND STAGE AT START OF ACUTE TESTS WITH H_2S

Test	Stage at start	Stage at collection	Sources	Date of collection	Temperature at collection, C	Method of collection
23	Juvenile	Juvenile	Lake Como	2/3/71	4	Trap
28	"	"	Cleveland Pond	8/2/72	24	Seine
29	"	Egg	" "	5/22/72	18	Natural spawning sites
31	"	"	" "	5/22/72	"	"
32	"	Juvenile	" "	9/27/72	15	Seine
33	"	"	" "	9/27/72	"	"

Day length was 12 hr. The water flow through the test chambers was 300 ml/min.

All tests consisted of one control and five H_2S concentrations supplied by diluters with "dipping-bird" dispensers. Eggs were taken off spawning tiles within 24 hr of deposition. Fry were hatched in the laboratory and juveniles were seined from ponds or reared in the laboratory.

Thirty-two acute tests were run in six series. One series was done on eggs, one on fry, one to determine effect of temperature, one to determine difference in response of wild and laboratory-reared fish, and two to compare acute lethal concentrations with chronic concentrations.

Acute Toxicity

Eggs—Six bioassays were run on fathead eggs (Table 3) at 23.8-24.2 C and oxygen of 5.6-6.0 mg/liter. The 96-hr LC50 varied between tests from 0.0190 to 0.0610 mg/liter, with a mean of 0.0350 mg/liter H_2S. LTC or time to hatch was attained in 5 to 8 days at 0.0190 to 0.0595 mg/liter with a mean of 0.0345 mg/liter H_2S.

Fry—Three tests on fry started within 24 hr of hatching were run at 24 C and 5.4-6.2 mg/liter O_2. The mean LC50 varied from 0.0136 at 24 hr to 0.0070 mg/liter H_2S at 96 hr. Mean LTC at 6 days was 0.0061 mg/liter H_2S. LC50 at 96 hr varied from 0.0066 to 0.0073 mg/liter H_2S and LTC from 0.0057 to 0.0066 mg/liter H_2S.

Juveniles at Various Temperatures—Bioassays were run at six temperatures: 6.5, 7.6, 10.0, 15.0, 20.2, and 25.0 C. LC50 at 96 hr ranged from 0.3800 mg/liter at 6.5 C to 0.0280 mg/liter H_2S at 25.0 C (Table 4). Fish used for test at 6.5 C were taken in January when lake water was 4 C. Other tests were made on fish caught when lake temperature approximated test temperatures.

Table 3. ACUTE TOXICITY OF H_2S (LC50 AND LTC) TO FATHEAD MINNOW EGGS AND FRY[a]

Test	Days to test[b]	Mean length, mm	Fish or eggs/ chamber	Tempera-ture, C	O_2, mg/l	LC50, mg/l H_2S 24 hr	48 hr	72 hr	96 hr	LTC[c] (days)
					Eggs – Duluth stock					
30	0	—	25	24.0	5.6	—	—	—	0.0205	0.0205 (5)
34	0	—	38	23.8	5.8	—	—	—	0.0440	0.0445 (5)
36	0	—	25	24.1	5.7	—	—	—	0.0190	0.0190 (4)
40	0	—	20	24.2	6.0	—	—	—	0.0285	0.0283 (6)
41	0	—	20	24.0	5.5	—	—	—	0.0610	0.0593 (8)
42	0	—	30	24.1	5.9	—	—	—	0.0370	0.0350 (8)
					$\bar{x}$ =	—	—	—	0.0350	0.0345
					Fry – Duluth stock					
43	0	5.6	20	24.1	5.4	0.0185	0.0130	0.0115	0.0069	0.0061 (6)
44	0	5.9	20	24.0	6.2	0.0087	0.0072	0.0063	0.0066	0.0066 (6)
45	0	5.6	20	24.0	6.2	—	0.0096	0.0081	0.0075	0.0057 (6)
					$\bar{x}$ =	0.0136	0.0099	0.0086	0.0070	0.0061

[a] pH: 7.9.
[b] Days elapsed between collection of specimens and start of test.
[c] Total hatch.

TABLE 4. ACUTE TOXICITY OF H_2S (LC50 AND LT50) TO [illegible] [illegible] JUVENILE [illegible] AT VARIOUS TEMPERATURES[a]

Test	Days to test[b]	Mean fish length, mm	Fish per chamber	Temperature, C	O_2 mg/l	LC50, mg/l H_2S			
						24 hr	48 hr	72 hr	96 hr
21	6	47.0	40	6.5	5.2	-	-	-	0.5800
20	7	50.0	40	7.6	6.3	0.7100	0.6500	0.6000	0.5200
19	7	30.0	40	10.0	6.0	-	-	0.2400	0.1500
18	7	34.0	40	15.0	6.0	-	0.0600	0.0570	0.0570
17	7	28.5	40	20.2	5.7	-	0.0420	0.0370	0.0320
16	6	28.0	40	25.0	6.1	0.0340	0.0310	0.0300	0.0280

[a] pH 7.9.

[b] Days elapsed between collection of specimen and start of test.

Acute Tests with Field and Laboratory-reared Fish—Two tests with field-reared juvenile stock and two with fish of the same stock reared from eggs in the laboratory were run at [illegible] C (Table 5) to determine whether laboratory conditions during previous life had a significant influence on sensitivity to H_2S. The mean 96-hr LC50 for field-reared fish was [illegible] mg/liter and the LTC was [illegible] mg/liter and for laboratory-reared fish was [illegible] and [illegible] mg/liter H_2S, respectively.

Juvenile Acute Tests for Chronic Test Comparison—Nine tests were run on juvenile fish of wild stock and four on Duluth laboratory stock to determine LC50's (Table 6) for comparison with chronic tests at low concentrations of H_2S. The acute tests were run at the time chronic tests were started. The mean 96-hr LC50 of six tests on 7 to 10 wild fish at 19.9 to 20.0 C was 0.0367 mg/liter H_2S.

Four tests run on Duluth stock were somewhat less than half as tolerant as the wild stock at 96 hr. The tests were run at 19.8 to 22.2 C (Table 6). The mean 96-hr LC50 was 0.0162 mg/liter and the mean LTC was 0.0120 mg/liter H_2S. Variation in LC50's between tests did not exceed 0.006 mg/liter at 96 hr and did not exceed 0.003 mg/liter at LTC.

CHRONIC BIOASSAY

Experimental Design

Five chronic tests were conducted on fathead minnows and were started as eggs or juveniles (Table 7). Three tests (chronic 2, 3, and 5) were with wild juvenile fish. Test 4 was started with sac fry of Duluth stock eggs. The chronic tests, designated as chronic 6_1 and 6_2, were continuous for two generations and initially employed sac fry of Duluth stock eggs. Tests 2, 3, 4, 6_1, and 6_2 were run to determine no-effect concentration and 5 to check effects of fish density.

Diluters were those described for use with sodium sulfide and toxicant

Table 5. ACUTE TOXICITY OF H_2S (LC50 AND LTC) TO FIELD-REARED AND LABORATORY-REARED JUVENILE FISH AT 20 C[a]

Test	Days to test[b]	Mean fish length, mm	Number of fish/chamber	Temperature, C	O_2, mg/l	LC50, mg/l H_2S 24 hr	48 hr	72 hr	96 hr	LTC (days)
				Laboratory-reared						
29	79	20.0	20	24.0	5.2	0.0270	0.0260	0.0192	0.0179	0.0175 (8)
31	133	34.0	20	24.0	5.4	0.0300	0.0280	0.0245	0.0245	0.0220 (7)
					$\bar{x}$ =	0.0285	0.0270	0.0218	0.0212	0.0198
				Field-reared						
32	34	26.0	20	23.8	5.4	0.0287	0.0226	0.0220	0.0215	0.0210 (8)
33	46	32.0	20	24.1	5.3	0.0310	0.0280	0.0203	0.0200	0.0197 (7)
					$\bar{x}$ =	0.0298	0.0253	0.0212	0.0208	0.0204

[a] pH: 7.9.

[b] Days elapsed between collection of specimen and start of test.

Table 6. ACUTE TOXICITY OF H_2S (LC50 AND LTC) TO FATHEAD MINNOWS OF WILD AND DULUTH STOCK[a]

(Tests Run to Provide Base for Application Factor)

Test	Days to test[b]	Mean fish length, mm	Fish per chamber	Temperature, C	O_2, mg/l	LC50, mg/l H_2S 48 hr	72 hr	96 hr	LTC(days)
				Wild stock					
4	39	35.1	7	20.0	6.5	-	0.0390	0.0330	-
7	67	34.9	8	20.0	6.1	0.0420	0.0390	0.0380	-
8	74	36.2	8	19.9	6.1	-	0.0400	0.0390	-
9	35	38.3	10	19.9	6.0	0.0430	0.0390	0.0340	-
11	70	34.0	10	19.9	6.0	-	-	0.0350	-
12	19	-	10	20.0	6.0	-	-	0.0390	-
					$\bar{x}$ =	0.0425	0.0392	0.0367	-
				Duluth stock					
22	-	34.0	15	19.8	5.5	0.2000	0.0170	0.0160	-
24	-	36.0	18	22.2	5.8	-	0.0210	0.0180	0.0130(8)
25	-	39.0	16	22.0	6.1	-	-	0.0180	0.0120(10)
26	-	32.0	11	22.0	6.0	-	-	0.0127	0.0109(9)
					$\bar{x}$ =	0.2000	0.0190	0.0162	0.0120

[a] pH 7.9.

[b] Days elapsed between collection of specimen and start of test.

Table 7. [illegible]

[illegible]

Test	Stage at start	Source	Date of collection	Water temp. at collection, C	Method of collection
2	Juvenile	Cleveland Pond	12/23/68	4	Trap
3	Juvenile	Cleveland Pond	8/20/69	22	Seine
4	Sac fry	Duluth stock	11/12/70	22	Spawning tiles
5	Juvenile	Lake Como	12/16/71	4	Trap
6_1	Sac fry	Duluth stock	3/24/71	22	Spawning tiles
6_2	Juvenile	Duluth stock	10/18/71	23	Spawning tiles

[illegible] [illegible] in the [illegible] [illegible]. In chronic tests 2 and 3 five [illegible] units were divided into ten sections (a to j) so that water flowed from [illegible] through section "a" into "j". Each section measured 15 x 40 x 31 cm with a water depth of 21 cm and a total volume of 12.6 liters. Concentrations of H_2S reported were based on analyses of water from each section (Table 8). The 20-liter tanks described above for the acute tests were used for chronic tests 4, 5, 6_1, and 6_2. In tests 4, 6_1, and 6_2 there were three replications (a, b, c) each with a separate diluter. In chronic test 5 there were five replications on the control and each of the three concentrations of H_2S (Table 9). In chronic test 4 there were three replications of the control and the four H_2S concentrations (Table 10).

In chronic tests 6_1 and 6_2, run consecutively in the same diluter, the three replications (Rep a, b, c) were run in parallel on separate levels of the diluter bank. On a fourth level another series designated as Fry Bank was operated for 80 days after spawning started in test 6_1 to hatch eggs and rear fry (Table 11). After 80 days these juveniles were placed in the three replications of chronic test 6_2. When spawning started in test 6_2 eggs were placed in the Fry Bank of test 6_2 to hatch and the fry were held for the 80-day period (Table 12).

Chronic tests 2 and 6_2 were started with 20 fish per chamber and chronic 4 with 15 fish per chamber. Fish were randomly removed from all tanks in each test just prior to spawning so all tanks contained 10 fish per chamber unless prior mortality reduced the number to less than 10. Chronic 3 and 6_1 were started with 10 fish per tank and were not thinned. Chronic test 5 was started with various numbers of fish from 4 to 19 to determine the effect of density on survival and growth. Controls and two series of H_2S concentrations were employed (target concentrations 1 and 2).

The day length was varied with seasonal changes in all tests. Day

TABLE [illegible]. TEST CONDITIONS IN CHRONIC [illegible] [illegible] TESTS 2 AND 3 STARTED WITH WILD STOCK JUVENILES[a]

Chronic 2: Tank	5a	2a	4a	3a	1a
$\bar{x}$ H_2S concentration (mg/1)	—	0.0019	0.0070	0.0093	0.0126
H_2S std. dev. (mg/1)	—	0.0010	0.0038	0.0046	0.0054
$\bar{x}$ pH	7.83	7.89	7.87	7.90	7.94
$\bar{x}$ temperature (C)	20.1	20.0	20.1	20.1	19.9
$\bar{x}$ dissolved O_2 (mg/1)	6.60	6.60	6.60	6.60	6.60
$\bar{x}$ total alkalinity (mg/1)	—	—	—	—	—
Tank	5b	2b	4b	3b	1b
$\bar{x}$ H_2S concentration (mg/1)	--	0.0015	0.0048	0.0060	0.0080
H_2S std. dev. (mg/1)	—	0.0010	0.0028	0.0029	0.0033
$\bar{x}$ pH	7.85	7.86	7.85	7.87	7.94
$\bar{x}$ temperature (C)	20.1	19.8	20.1	20.0	20.0
$\bar{x}$ dissolved O_2 (mg/1)	6.60	6.60	6.60	6.60	6.60
$\bar{x}$ total alkalinity (mg/1)	--	--	--	--	--

Chronic 3: Tank	1a	5a	4a	3a	2a
$\bar{x}$ H_2S concentration (mg/1)	—	0.0005	0.0024	0.0068	0.0198
H_2S std. dev. (mg/1)	—	0.0006	0.0012	0.0027	0.0078
$\bar{x}$ pH	7.67	7.69	7.69	7.73	7.84
$\bar{x}$ temperature (c)	21.2	21.3	21.3	21.3	21.2
$\bar{x}$ dissolved O_2 (mg/1)	7.52	8.15	8.25	8.20	8.23
$\bar{x}$ total alkalinity (mg/1)	194	194	194	194	194
Tank	1b	5b	4b	3b	2b
$\bar{x}$ H_2S concentration (mg/1)	—	0.0004	0.0010	0.0022	0.0078
H_2S std. dev. (mg/1)	—	0.0004	0.0008	0.0012	0.0034
$\bar{x}$ pH	7.67	7.67	7.69	7.71	7.85
$\bar{x}$ temperature (C)	21.3	21.3	21.3	21.3	21.2
$\bar{x}$ dissolved O_2 (mg/1)	7.99	7.61	8.10	7.96	8.23
$\bar{x}$ total alkalinity (mg/1)	194	194	194	194	194

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[a] 20 fish started in each chamber and thinned to 10 before first spawning.

Length of daylight in tests 2 and 3 was synchronized with that of St. Paul, Minnesota. The day length for [illegible]. [illegible] was used in tests 3, 4, 6_1, and 6_2 as recommended by the National Water Quality Laboratory at Duluth. The temperature was varied seasonally in tests 2 and 3 and was held constant in tests 4, 5, 6_1, and 6_2.

A substrate for spawning was provided by half cylinders cut from 3-inch lengths of cement-asbestos pipe and placed in the test chambers with the convex side up.

Eggs were incubated in Nitex screen-bottomed glass cylinders which were continuously moved up and down 1.5 cm in a 10-sec cycle. The oscillation was provided by attachment of the cylinder to a variable speed gear box and an off-set cam wheel.

All spawned eggs from each chamber were counted. From at least one of each three spawnings in each test chamber of test 6_1 and 6_2 a random subsample of 50 eggs was incubated to hatch to determine percentage survival to hatch. Eggs from each treatment were hatched in that treatment and in control water.

If fish appeared stressed by diagnosed bacterial disease they were treated with tetracycline or neomycin at the rate of 20 mg/liter for 3 days with continuous inflow of freshwater without the H_2S used for treatment. Combinations of formalin and methylene blue or copper sulfate were used for protozoan infections.

After reaching 20 mm total length, fish were weighed in water at 4-week intervals until sexual maturity. All the fish of a single test chamber were weighed in water as a group.

Newly hatched fry were fed a suspension of mashed hard-boiled egg yolk at least three times per day. When a size of about 8 mm total length

Table 9. [illegible]

Control Tank	3	2	4	5	1
No. fish/tank	4	6	10	14	19
$\bar{x}$ H_2S concentration (mg/l)	-	-	-	-	-
H_2S std. dev. (mg/l)	-	-	-	-	-
$\bar{x}$ pH	7.76	7.75	7.75	7.74	7.72
$\bar{x}$ temperature (C)	19.6	19.[illegible]	19.6	19.5	19.8
$\bar{x}$ dissolved O_2 (mg/l)	9.54	8.86	9.52	8.81	8.92
$\bar{x}$ total alkalinity (mg/l)	203	203	203	203	203
Target concentration 1 Tank	5	4	3	1	2
No. fish/tank	4	6	10	14	19
$\bar{x}$ H_2S concentration (mg/l)	0.0014	0.0018	0.0016	0.0019	0.0017
H_2S std. dev. (mg/l)	0.0008	0.0010	0.0008	0.0011	0.0009
$\bar{x}$ pH	7.78	7.76	7.74	7.75	7.72
$\bar{x}$ temperature (C)	19.9	19.8	19.9	20.0	19.8
$\bar{x}$ dissolved O_2 (mg/l)	8.97	8.77	8.31	7.87	7.71
$\bar{x}$ total alkalinity (mg/l)	203	203	203	203	203
Target concentration 2 Tank	4	2	5	3	1
No. fish/tank	4	6	10	14	19
$\bar{x}$ H_2S concentration (mg/l)	0.0029	0.0052	0.0038	0.0046	0.0038
H_2S std. dev. (mg/l)	0.0025	0.0028	0.0024	0.0024	0.0021
$\bar{x}$ pH	7.77	7.75	7.75	7.75	7.72
$\bar{x}$ temperature (C)	21.9	21.5	21.6	21.5	21.5
$\bar{x}$ dissolved O_2	8.80	8.52	8.44	8.29	6.79
$\bar{x}$ total alkalinity (mg/l)	203	203	203	203	203

Table [illegible] continued. TEST CONDITIONS IN CHRONIC HYDROGEN SULFIDE TEST 5
[illegible] WITH [illegible] JUVENILES

Target concentration 2 Tank	1	4	2	3	5
No. fish/tank	4	6	10	14	19
$\bar{x}$ H_2S concentration (mg/1)	0.0094	0.0111	0.0070	0.0099	0.0068
H_2S std. dev. (mg/1)	0.0083	0.0062	0.0044	0.0077	0.0045
$\bar{x}$ pH	7.76	7.78	7.77	7.74	7.77
$\bar{x}$ temperature (C)	20.7	20.9	21.1	20.8	20.7
$\bar{x}$ dissolved O_2 (mg/1)	8.57	8.09	7.97	7.47	6.83
$\bar{x}$ total alkalinity (mg/1)	203	203	203	203	203

[illegible]

Replicate a	Tank 5a	4a	3a	1a	2a
$\bar{x}$ H_2S concentration (mg/l)	—	0.0010	0.0022	0.0052	—
H_2S std. dev. (mg/l)	—	0.0007	[illegible]	[illegible]	—
$\bar{x}$ pH	7.68	7.64	7.65	7.67	—
$\bar{x}$ temperature (C)	23.8	23.9	23.9	23.5	—
$\bar{x}$ dissolved O_2 (mg/l)	8.66	8.73	[illegible]	7.99	—
$\bar{x}$ total alkalinity (mg/l)	208	208	208	208	—

Replicate b	Tank 5b	4b	3b	1b	2b
$\bar{x}$ H_2S concentration (mg/l)	—	0.0006	0.0016	0.0045	0.0078
H_2S std. dev. (mg/l)	—	0.0004	0.0019	0.0037	0.0063
$\bar{x}$ pH	7.76	7.74	7.72	7.70	7.78
$\bar{x}$ temperature (C)	23.0	22.9	22.8	22.5	22.7
$\bar{x}$ dissolved O_2 (mg/l)	8.99	8.33	7.92	7.77	7.82
$\bar{x}$ total alkalinity (mg/l)	208	208	208	208	208

Replicate c	Tank 5c	4c	3c	1c	2c
$\bar{x}$ H_2S concentration (mg/l)	—	0.0010	0.0021	0.0046	0.0102
H_2S std. dev. (mg/l)	—	0.0007	0.0024	0.0048	0.0076
$\bar{x}$ pH	7.69	7.65	7.63	7.67	7.67
$\bar{x}$ temperature (C)	23.9	24.1	24.2	23.8	24.0
$\bar{x}$ dissolved O_2 (mg/l)	8.56	8.16	8.06	7.45	7.46
$\bar{x}$ total alkalinity (mg/l)	208	208	208	208	208

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[a] Fifteen sac fry per chamber at start and thinned to 10 fish prior to first spawning.

Table 11. TEST CONDITIONS IN FATHEAD MINNOW CHRONIC TEST C_1 STARTED WITH DULUTH STOCK SAC FRY[a]

Replicate a Tank	5a	4a	3a	1a	2a
$\bar{x}$ H_2S concentration (mg/l)	—	0.0004	0.0012	0.0033	0.0063
H_2S std. dev. (mg/l)	—	0.0003	0.0004	0.0008	0.0019
$\bar{x}$ pH	7.75	7.73	7.72	7.72	7.77
$\bar{x}$ temperature (C)	23.1	23.1	23.1	22.9	23.0
$\bar{x}$ dissolved O_2 (mg/l)	7.14	6.81	6.48	6.39	6.31
$\bar{x}$ total alkalinity (mg/l)	214	214	214	214	214
Replicate b Tank	5b	4b	3b	1b	2b[b]
$\bar{x}$ H_2S concentration (mg/l)	—	0.0004	0.0011	0.0026	—
H_2S std. dev. (mg/l)	—	0.0003	0.0004	0.0010	—
$\bar{x}$ pH	7.76	7.77	7.73	7.77	—
$\bar{x}$ temperature (C)	22.7	22.7	22.7	22.5	—
$\bar{x}$ dissolved O_2 (mg/l)	7.39	7.27	7.07	7.18	—
$\bar{x}$ total alkalinity (mg/l)	214	214	214	214	—
Replicate c Tank	5c	4c	3c	1c	2c
$\bar{x}$ H_2S concentration (mg/l)	—	0.0005	0.0012	0.0033	0.0068
H_2S std. dev. (mg/l)	--	0.0003	0.0004	0.0011	0.0020
$\bar{x}$ pH	7.75	7.74	7.73	7.74	7.76
$\bar{x}$ temperature (C)	23.1	23.2	23.2	22.9	23.1
$\bar{x}$ dissolved O_2 (mg/l)	6.98	6.74	6.62	6.49	6.48
$\bar{x}$ total alkalinity (mg/l)	214	214	214	214	214
Fry tank Tank	5d	4d	3d	1d	2d
$\bar{x}$ H_2S concentration (mg/l)	—	0.0004	0.0007	0.0026	0.0041
H_2S std. dev. (mg/l)	--	0.0002	0.0003	0.0011	0.0011
$\bar{x}$ pH	7.81	7.83	7.84	7.85	7.89
$\bar{x}$ temperature (C)	22.5	22.4	22.4	22.2	22.3
$\bar{x}$ dissolved O_2 (mg/l)	7.07	7.18	7.03	6.68	6.95
$\bar{x}$ total alkalinity (mg/l)	214	214	214	214	214

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[a] Started with 10 fry.
[b] Fish all killed by low O_2.

Table 13. TEST CONDITIONS IN FATHEAD MINNOW CHRONIC TEST 6_2 STARTED WITH FISH FROM CHRONIC 6_1 SPAWNINGS[a]

Replicate a	Tank	5a	4a	3a	1a	2a
$\bar{\bar{x}}$ H_2S concentration (mg/1)		—	0.0008	0.0014	0.0035	0.0060
H_2S std. dev. (mg/1)		—	0.0005	0.0007	0.0016	0.0035
$\bar{\bar{x}}$ pH		7.68	7.65	7.67	7.67	7.67
$\bar{\bar{x}}$ temperature (C)		24.1	24.2	24.2	24.0	24.1
$\bar{\bar{x}}$ dissolved O_2 (mg/1)		6.78	6.35	6.44	6.31	6.17
$\bar{\bar{x}}$ total alkalinity (mg/1)		201	201	201	201	201
Replicate b	Tank	5b	4b	3b	1b	2b
$\bar{\bar{x}}$ H_2S concentration (mg/1)		—	0.0006	0.0012	0.0032	0.0054
H_2S std. dev. (mg/1)		—	0.0005	0.0005	0.0016	0.0025
$\bar{\bar{x}}$ pH		7.71	7.71	7.71	7.69	7.73
$\bar{\bar{x}}$ temperature (C)		23.6	23.6	23.7	23.5	23.6
$\bar{\bar{x}}$ dissolved O_2 (mg/1)		6.83	6.79	6.83	6.46	6.58
$\bar{\bar{x}}$ total alkalinity (mg/1)		201	201	201	201	201
Replicate c	Tank	5c	4c	3c	1c	2c
$\bar{\bar{x}}$ H_2S concentration (mg/1)		—	0.0007	0.0014	0.0044	0.0074
H_2S std. dev. (mg/1)		—	0.0005	0.0005	0.0028	0.0030
$\bar{\bar{x}}$ pH		7.69	7.66	7.66	7.68	7.68
$\bar{\bar{x}}$ temperature (C)		24.3	24.3	24.3	24.0	24.3
$\bar{\bar{x}}$ dissolved O_2 (mg/1)		6.76	6.56	6.37	6.52	6.21
$\bar{\bar{x}}$ total alkalinity (mg/1)		201	201	201	201	201
Fry tank	Tank	5d	4d	3d	1d	2d
$\bar{\bar{x}}$ H_2S concentration (mg/1)		—	0.0007	0.0009	0.0018	0.0050
H_2S std. dev. (mg/1)		—	0.0004	0.0005	0.0010	0.0023
$\bar{\bar{x}}$ pH		7.79	7.74	7.80	7.81	7.83
$\bar{\bar{x}}$ temperature (C)		24.2	24.1	24.1	24.0	24.9
$\bar{\bar{x}}$ dissolved O_2 (mg/1)		7.41	7.45	7.26	7.14	6.88
$\bar{\bar{x}}$ total alkalinity (mg/1)		201	201	201	201	201

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[a] Test started with 20 80-day juveniles from spawning in test 6_1.

was attained, finely pulverized dry Glencoe #1 fry granules were added to the diet, at least twice per day. At a size of about 10 mm, newly hatched brine shrimp were added twice per day. At 15 mm, feeding of egg-yolk suspension was stopped and a mixture of finely blended hard-boiled eggs and lettuce was substituted. At a size of about 30 mm the #1 fry granules were fed without pulverizing.

All tanks were illuminated with incandescent lights located about 30 cm above the water surface. Bulbs varied from 40 to 100 watts depending on the life history stage. For egg incubation and the early fry stages 40 watt bulbs were used, and by the time of sexual maturity the wattage was gradually increased to 100.

Survival in Chronic Exposure

Chronic tests 2 and 3 were started with wild stock juveniles (Table 13). Test 2 ran for 191 and test 3 for 345 days. After 51 days in both series of test 2, survival declined in control and all test concentrations of H_2S. At 0.0093 mg/liter H_2S in series a, survival was 61% with 93% in control at 79 days and at 191 days was 6% and 65%, respectively.

In series b, survival through 79 days was essentially the same as controls in all H_2S treatments except at 0.0080 mg/liter where it was substantially lower. Excessive loss in controls made comparisons doubtful for the remainder of the test.

Chronic 3, series a, had reduced survival at 0.0198 mg/liter H_2S by the 37th day and thereafter but at lower concentrations survival was similar or better than controls. Survival in series b with a maximum H_2S concentration of 0.0078 mg/liter was not lower than control in any concentration.

Chronic 4, with three replications (a,b,c) was started with Duluth stock sac fry and continued for 84 days (Table 14). Maximum concentration

Table 13. SURVIVAL OF WILD STOCK FATHEAD MINNOWS WITH LONG-TERM EXPOSURE TO H_2S IN CHRONIC TESTS 2 AND 3
(expressed as percentage)

Test	Exposure, days	H_2S concentration, mg/1				
Chronic 2		Control	0.0019	0.0070	0.0093	0.0126
series a	24	100	90	100	100	91
	51	100	84	75	61	0
	79	93	84	75	61	0
	107	93	84	75	50	0
	135	93	84	75	50	0
	163	86	84	75	16	0
	191	65	76	68	6	0
series b		Control	0.0015	0.0048	0.0060	0.0080
	24	100	95	100	100	94
	51	100	90	100	96	59
	79	95	84	90	96	59
	107	85	84	86	91	59
	135	77	76	86	82	53
	163	68	67	86	82	35
	191	34	42	69	73	24
Chronic 3		Control	0.0005	0.0024	0.0068	0.0198
series a	9	100	100	100	100	70
	37	80	100	100	89	20
	66	60	100	90	67	20
	177	60	90	90	67	20
	345	60	90	90	67	10

Table 13 continued. SURVIVAL OF WILD STOCK FATHEAD MINNOWS WITH LONG-TERM EXPOSURE TO H_2S IN CHRONIC TESTS 2 AND 3

(expressed as percentage)

Test	Exposure, days	H_2S concentration, mg/l				
		Control	0.0004	0.0010	0.0022	0.0078
Chronic 3						
series h	9	89	100	100	100	100
	37	67	89	89	80	60
	66	67	89	78	80	60
	177	56	89	78	80	50
	345	56	89	67	70	50

Table 14. SURVIVAL OF DULUTH STOCK FATHEAD MINNOWS STARTED AS SAC FRY WITH LONG-TERM EXPOSURE TO H_2S IN CHRONIC TEST 4 (expressed as percentage)

Test	Exposure, days	H_2S concentration, mg/l				
Series *a*		Control	0.0010	0.0022	0.0052	—[a]
	28	100	100	100	100	—
	56	100	100	93	100	—
	84	100	100	93	100	—
Series *b*		Control	0.0006	0.0016	0.0045	0.0078
	28	100	100	100	100	100
	56	100	87	100	80	67
	84	100	87	100	80	67
Series *c*		Control	0.0010	0.0021	0.0046	0.0102
	28	100	100	100	100	100
	56	100	93	100	100	20
	84	100	93	100	100	20

[a] Test fish accidentally killed by low pH.

in series a was 0.0032 mg/liter H_2S and no difference from controls was noted in any H_2S concentration. Survival in series b after 56 days was lower than controls in 0.0045 and 0.0078 mg/liter H_2S, but no further loss occurred up to 84 days. In series c, survival was substantially less than controls only at a concentration of 0.0102 mg/liter H_2S.

Chronic test 6_1 with three replications was started with Duluth stock sac fry and run for 297 days (Table 15). In the three replications survival was similar to that in controls except at 0.0063 and 0.0068 mg/liter H_2S where survival was reduced to 60% and 40%, respectively, by the end of the test.

Mortality of eggs and fry from adults in 6_1 held in series d (Fry Bank) for 80 days was not consistently different from controls in any H_2S concentration up to 0.0041 mg/liter.

Chronic test 6_2 was started with fish derived from spawning in test 6_1, series d, and held for 80 days at the same nominal concentrations. In test 6_2 run with the same nominal concentrations, significant mortality occurred above 0.0032 mg/liter H_2S (Table 16). At 0.0080 mg/liter in series a, survival was 71% of control after 196 days; in series b, 76% of control at 0.0054 mg/liter after 274 days; and in series c, 52% of control at 0.0074 mg/liter after 274 days. Maximum mortality at the highest levels was reached in 56 to 140 days and did not increase thereafter. Mortality of eggs and fry generated in test 6_2 and held for 80 days at same nominal concentrations (series d) varied from controls substantially at 0.0050 mg/liter and to a lesser degree at 0.0018 mg/liter H_2S.

Chronic 5 was conducted with three concentrations of H_2S plus one control with 4 to 19 juveniles fish per tank (Table 17) for 112 days. Fish were distributed on the basis of a random numbers table. The number of fish in the tanks did not affect the survival. At 0.0092 mg/liter

Table 15. SURVIVAL OF DULUTH STOCK FATHEAD MINNOWS IN CHRONIC TEST 6_1
STARTED AS SAC FRY WITH LONG-TERM EXPOSURE TO H_2S
(expressed as percentage)

Test	Exposure, days	H_2S concentration, mg/1				
Series a		Control	0.0004	0.0012	0.0033	0.0063
	28	100	100	100	90	70
	56	100	100	100	90	70
	84	100	100	100	90	70
	112	100	100	100	90	70
	140	100	90	100	90	70
	168	100	90	100	90	70
	196	100	90	100	90	70
	224	100	90	100	90	60
	252	100	90	100	90	60
	280	100	90	100	90	60
	297	100	90	100	90	60
Series b		Control	0.0004	0.0011	0.0026	—[a]
	28	100	90	80	100	—
	56	100	90	80	100	—
	84	100	90	80	100	—
	112	100	90	80	100	—
	140	100	90	80	100	—
	168	100	90	80	100	—
	196	100	90	80	100	—
	224	100	90	80	100	—
	252	100	80	80	100	—
	280	90	80	80	100	—
	297	90	80	80	100	—

Table 15 continued. SURVIVAL OF DULUTH STOCK FATHEAD MINNOWS IN CHRONIC TEST 6_1 STARTED AS SAC FRY WITH LONG-TERM EXPOSURE TO H_2S (expressed as percentage)

Test	Exposure, days	H_2S concentration, mg/l				
Series *c*		Control	0.0005	0.0012	0.0033	0.0068
	28	100	100	100	100	70
	56	100	100	100	100	70
	84	100	100	100	100	70
	112	100	100	100	100	70
	140	100	100	100	100	70
	168	100	100	100	100	70
	196	100	100	100	100	70
	224	100	100	100	100	70
	252	100	100	100	100	60
	280	100	100	100	90	50
	297	100	100	100	90	40
Series *d*[b]		Control	0.0004	0.0007	0.0026	0.0041
	80	55	62	56	61	78

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[a] Test fish accidentally killed by low O_2.

[b] Series *d* started with eggs from adults reared in 6_1.

Table 16. SURVIVAL OF FATHEAD MINNOWS STARTED AS JUVENILES FROM CHRONIC TEST C1 DONE WITH LONG-TERM EXPOSURE TO H_2S (expressed as percentage)

Test	Exposure,[a] days	H_2S concentration, mg/l				
		Control	0.0002	0.0014	0.0035	0.0060
Chronic C_2 series a	28	100	100	100	100	100
	56	100	100	100	100	71
	84	100	100	100	100	71
	112	100	100	100	100	71
	140	100	100	100	100	71
	168	100	100	100	100	71
	196	100	100	100	100	71
	224	100	100	100	100	0[b]
	274	100	100	100	100	0
		Control	0.0004	0.0012	0.0033	0.0054
series b	28	100	100	100	100	100
	56	100	100	100	100	95
	84	100	100	100	90	95
	112	100	100	100	90	95
	140	100	100	100	90	76
	168	100	100	100	90	76
	196	100	100	100	90	76
	224	100	100	100	90	76
	274	100	90	90	80	76

Table 16 continued. SURVIVAL OF FATHEAD MINNOWS STARTED AS JUVENILES FROM CHRONIC TEST 6_1 EGGS WITH LONG-TERM EXPOSURE TO H_2S (expressed as percentage)

Test	Exposure, days	H_2S concentration, mg/l				
		Control	0.0007	0.0014	0.0046	0.0074
Chronic 6_2 series c	28	100	100	100	100	86
	56	100	100	100	100	86
	84	100	100	100	100	52
	112	100	100	100	100	52
	140	100	90	100	100	52
	168	100	90	100	—[c]	52
	196	100	90	100	—	52
	224	100	90	100	—	52
	274	90	90	80	—	52
		Control	0.0007	0.0009	0.0018	0.0030
series d	680	100	79	100	85	64

[a] Exposure times do not include 680-day period of treatment in series d of 6_1.

[b] Fish lost to disease.

[c] Fish lost to low O_2.

Table 17. SURVIVAL OF WILD STOCK JUVENILE FATHEAD MINNOWS WITH DIFFERENT CONCENTRATIONS OF FISH AND H_2S IN CHRONIC TEST 5 (expressed as percentage)

Test	Exposure, days	Number of fish/tank 4	6	10	14	19
Control	28	100	100	100	100	100
	56	100	100	100	100	100
	84	100	100	100	100	100
	112	100	100	100	100	100
0.0017 mg/1 H_2S	28	100	100	100	100	100
	56	100	100	100	100	100
	84	100	100	100	100	100
	112	100	100	100	100	100
0.0041 mg/1 H_2S	28	100	100	100	100	100
	56	100	100	100	100	100
	84	100	100	100	100	100
	112	100	100	100	100	100
0.0092 mg/1 H_2S	28	100	100	100	100	95
	56	100	100	90	100	95
	84	50	100	90	100	95
	112	50	83	90	93	95

H_2S some loss occurred with the highest percentage loss in the tank with the smallest number of fish.

Growth

Growth of wild stock juvenile minnows in various concentrations of H_2S was observed in chronic 2 and 3 (Table 18). After 107 days in chronic 2, series a, growth was adversely affected at 0.0093 mg/liter; and in series b, at 0.0080 mg/liter H_2S. In series a of chronic 3, fish grew approximately 33% less at 0.0198 mg/liter H_2S than in control in 121 days but no reduction occurred at 0.0068 mg/liter. In series b of chronic 3, percentage increment was not appreciably different from control at 0.0078 mg/liter H_2S. At lower levels growth was greater than in the control.

Chronic 4 was started with Duluth stock sac fry and carried for 84 days (Table 19). At termination in series a, growth was greater than controls in all H_2S concentrations. In series b, growth was the same or greater than control with concentrations of H_2S up to 0.0045 mg/liter but at 0.0078 mg/liter was lower. In series c, growth was retarded at 0.0102 mg/liter H_2S.

Chronic 5 was conducted to determine the effect of fish density in test chambers on the growth rate in H_2S (Table 20). Three concentrations of H_2S and one control with five densities of fish (4 to 19) were tested. With 6 to 19 fish there were no consistent differences associated with fish density in the various treatments. It was concluded that within the fish density range tested, reaction to H_2S was not affected by number of fish per chamber.

In chronic 6_1 growth in the three series (a,b,c) was not closely related to H_2S concentration except during the first part of the exposure (Table 21). After 112 days at 0.0063 mg/liter H_2S in series a, growth was less than control but in series c at 0.0068 mg/liter H_2S, there was little

Table 18. GROWTH OF WILD STOCK FATHEAD MINNOWS AT VARIOUS CONCENTRATIONS OF H_2S IN CHRONIC TESTS 2 AND 3 (expressed as mean weight in grams)

Test	Exposure, days	H_2S concentration, mg/l				
Chronic 2		Control	0.0019	0.0070	0.0093	0.0126
series *a*	0	0.893[a]	0.893	0.893	0.893	0.893
	51	1.347	1.256	1.225	1.096	1.023
	79	2.162	1.875	1.981	1.493	-
	107	2.227	1.989	2.242	1.866	-
series *b*		Control	0.0015	0.0048	0.0060	0.0080
	0	0.893[a]	0.893	0.893	0.893	0.893
	51	1.100	1.200	1.165	1.342	1.138
	79	1.784	1.800	1.669	1.825	1.729
	107	1.943	1.826	1.866	1.945	1.786
Chronic 3						
series *a*		Control	0.0005	0.0024	0.0068	0.0198
	0	0.362	0.343	0.441	0.419	0.351
	9	0.525	0.664	0.757	0.657	0.564
	37	0.985	0.877	1.186	1.160	1.718
	66	1.970	1.640	2.050	1.990	1.090
	93	2.010	1.670	2.150	2.120	1.160
	121	2.110	1.740	2.210	2.240	1.390
series *b*		Control	0.0004	0.0010	0.0022	0.0078
	0	0.417	0.477	0.488	0.454	0.384
	9	0.635	0.830	0.980	0.849	0.538
	37	1.098	1.121	1.439	1.164	0.971
	66	1.560	2.290	2.400	1.980	1.620
	93	1.620	2.470	2.600	2.190	1.690
	121	1.770	2.480	2.630	2.260	1.820

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[a] Based on mean weight (g) of a random sample of the stock used to start test.

Table 19. GROWTH OF DULUTH STOCK FATHEAD MINNOWS STARTED AS EGGS IN CHRONIC TEST 4

(expressed as mean weight in grams)

Test	Exposure, days	H_2S concentration, mg/l				
Series *a*		Control	0.0010	0.0022	0.0052	—[a]
	56	0.416	0.392	0.474	0.386	—
	84	0.652	0.710	0.666	0.674	—
Series *b*		Control	0.0006	0.0016	0.0045	0.0078
	56	0.375	0.329	0.368	0.354	0.269
	84	0.710	0.708	0.690	0.818	0.519
Series *c*		Control	0.0010	0.0021	0.0046	0.0102
	56	0.421	0.379	0.381	0.337	0.165
	84	0.747	0.678	0.788	0.664	0.438

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[a] Accidentally killed by low pH.

Table 20. GROWTH OF JUVENILE FATHEAD MINNOWS IN CHRONIC TEST 5 WITH VARIED CONCENTRATIONS OF H_2S AND DIFFERENT NUMBERS OF FISH IN TEST CHAMBERS

(expressed as mean weight in grams)

Test	Exposure, days	Number of fish/tank 4	6	10	14	19
Control	28	0.76	0.88	0.80	0.88	0.91
	56	1.31	1.40	1.51	1.55	1.69
	84	1.80	2.03	2.17	2.14	2.38
	112	2.52	2.31	2.73	2.79	2.77
0.0017 mg/l H_2S	28	0.80	0.94	0.89	0.94	0.93
	56	1.45	1.64	1.71	1.98	1.71
	84	1.95	2.20	2.24	2.66	2.24
	112	2.65	2.76	2.86	3.27	2.69
0.0041 mg/l H_2S	28	0.96	0.85	0.91	0.83	0.87
	56	1.78	1.68	1.62	1.60	1.85
	84	2.16	2.50	1.98	2.03	2.32
	112	2.70	2.86	2.20	2.43	2.86
0.0092 mg/l H_2S	28	1.12	0.97	1.05	0.91	0.94
	56	1.64	1.41	1.71	1.66	2.02
	84	2.80	2.10	2.14	1.77	2.09
	112	3.65	2.48	2.83	2.18	2.47

difference from the control. In the second cycle of this experiment described as chronic 6_2 growth after 56 days was not uniformly related to H_2S concentration up to 0.0032 mg/liter. Again, early effects were overcome later.

Reproductive Success

Spawning success in chronic 6_1 started with Duluth sac fry was not changed consistently with H_2S concentrations as measured by number of eggs deposited per female or total number of spawnings (Table 22). During the reproductive period the total number of eggs per female varied from 180 to 2614 in 4 to 33 spawnings in different H_2S treatments. Mean number of spawnings per female varied from 1.25 to 13.50. Total eggs spawned per female fell off at 0.0068 mg/liter H_2S in series c because there was mortality during the progress of the experiment. Some treatments had larger numbers of eggs per female than the controls. In the second cycle, chronic 6_2 (Table 23), the number of eggs laid per female was smaller in most cases than in the first cycle but there was no consistent relationship between number of eggs spawned and H_2S concentration. At treatments of H_2S up to 0.0074 mg/liter in both chronic 6_1 and 6_2, no trends in spawning and egg deposition were related to increased concentrations of H_2S. During test 6_1 there was a delay of about 20 days in the start of spawning for all treatments as compared with the controls. For test 6_2 there was a delay of about 40 days for the highest H_2S levels but the other treatments were equal to the controls.

Time to Hatch

Eggs deposited in the three series (a, b, c) of chronic 6_1 and 6_2 were hatched in the same H_2S concentrations where they were deposited and some were removed from treatments and hatched in control water (Table 24). No variation in days to hatch attributable to H_2S concentration during hatch was noted.

Table 21. GROWTH OF DULUTH STOCK FATHEAD MINNOWS STARTED AS SAC FRY IN AN EXPERIMENT COVERING TWO GENERATIONS
(expressed as mean weight in grams)

Test	Exposure, days	H_2S concentration, mg/l				
Chronic 6_1						
series a		Control	0.0004	0.0012	0.0033	0.0063
	28	0.102	0.120	0.118	0.110	0.105
	56	0.445	0.411	0.398	0.339	0.337
	84	0.704	0.711	0.584	0.782	0.549
	112	1.335	1.237	1.094	1.281	0.985
series b		Control	0.0004	0.0011	0.0026	-[a]
	28	0.104	0.098	0.119	0.087	-
	56	0.429	0.400	0.386	0.368	-
	84	0.661	0.716	0.634	0.557	-
	112	1.114	1.130	1.229	1.207	-
series c		Control	0.0005	0.0012	0.0033	0.0068
	28	0.111	0.114	0.110	0.110	0.112
	56	0.497	0.452	0.364	0.482	0.394
	84	0.690	0.641	0.575	0.809	0.671
	112	1.274	0.978	0.908	1.067	1.230
Chronic 6_2 [b]						
series a		Control	0.0008	0.0014	0.0035	0.0080
	0	0.614	0.376[c]	0.630	0.543	0.436
	28	0.959	0.797	0.960	0.828	0.700
	56	1.188	1.165	1.233	1.054	0.653

Table 21 continued. GROWTH OF DULUTH STOCK FATHEAD MINNOWS STARTED AS SAC FRY IN AN EXPERIMENT COVERING TWO GENERATIONS
(expressed as mean weight in grams)

Test	Exposure, days	H_2S concentration, mg/l				
Chronic 6_2[b]						
series b		Control	0.0006	0.0012	0.0032	0.0054
	0	0.631	0.462[c]	0.688	0.536	0.386
	28	1.007	0.916	1.025	0.817	0.664
	56	1.376	1.354	1.267	1.105	0.962
series c		Control	0.0007	0.0014	0.0044	0.0074
	0	0.602	0.324[c]	0.628	0.319	0.352
	28	0.928	0.685	0.895	0.790	0.600
	56	1.183	0.983	1.166	1.078	0.875

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[a] Fish accidentally killed by low O_2.

[b] Exposure times do not include @ 80-day period of treatment in d series of 6_1.

[c] The smaller mean size at day 0 for the lowest concentration was probably due to overcrowding during exposure in the d series of 6_1 (about 50% more fry were accidentally placed in this chamber).

Table 22. SPAWNING SUCCESS, EGGS PER FEMALE AND NUMBER OF SPAWNINGS PER FEMALE IN DULUTH STOCK FATHEAD MINNOWS WITH CONTINUED EXPOSURE TO LOW LEVELS OF H_2S IN CHRONIC TEST 6_1

Test	H_2S concentration, mg/l				
Series *a*	Control	0.0004	0.0012	0.0033	0.0063
No. females	2	4	2	2	3
No. spawnings	27	22	9	19	20
Total eggs/tank	2591	2638	853	3023	4037
Eggs/spawning	96	120	95	159	202
Mean spawnings/female	13.50	5.50	4.50	9.50	10.50
Mean eggs/female	1296	660	426	1512	1346
Series *b*	Control	0.0004	0.0011	0.0026	--[a]
No. females	5	4	3	4	--
No. spawnings	44	33	16	33	--
Total eggs/tank	8503	10456	2417	7215	--
Eggs/spawning	193	317	151	219	--
Mean spawnings/female	8.80	8.25	5.33	8.25	--
Mean eggs/female	1701	2614	806	1804	--
Series *c*	Control	0.0005	0.0012	0.0033	0.0068
No. females	4	5	4	3	1
No. spawnings	21	25	5	8	4
Total eggs/tank	1900	4221	722	1591	399
Eggs/spawning	90	169	144	199	100
Mean spawnings/female	5.25	5.00	1.25	2.67	4.00
Mean eggs/female	475	844	180	530	399

[a] Fish all killed by low O_2.

Table 23. SPAWNING SUCCESS, EGGS PER FEMALE AND NUMBER OF SPAWNINGS PER FEMALE IN DULUTH STOCK FATHEAD MINNOWS WITH CONTINUED EXPOSURE TO LOW LEVELS OF H_2S IN CHRONIC TEST 6_2

Test	H_2S concentration, mg/l				
Series a	Control	0.0008	0.0014	0.0035	—[a]
No. females	7	4	6	4	—
No. spawnings	27	28	21	13	—
Total eggs/tank	3164	3625	2675	1336	—
Eggs/spawning	117	129	127	102	—
Mean spawnings/female	3.86	7.00	3.50	3.25	—
Mean eggs/female	452	906	445	334	—
Series b	Control	0.0006	0.0012	0.0032	0.0054
No. females	6	3	4	5	2
No. spawnings	27	11	3	23	2
Total eggs/tank	4301	603	482	3480	189
Eggs/spawning	159	55	160	151	94
Mean spawnings/female	4.50	3.67	0.75	4.60	1.00
Mean eggs/female	717	201	120	696	94
Series c	Control	0.0007	0.0014	—[b]	0.0074
No. females	8	7	6	—	3
No. spawnings	62	62	40	—	28
Total eggs/tank	11691	11588	3446	—	3774
Eggs/spawning	188	186	86	—	134
Mean spawnings/female	7.75	8.86	6.67	—	9.33
Mean eggs/female	1461	1655	574	—	1258

[a] Fish all killed by low O_2.

[b] All fish died of disease at start of spawning.

Table 24. NUMBER OF DAYS TO HATCH OF FATHEAD MINNOW EGGS DEPOSITED IN VARIOUS CONCENTRATIONS OF H_2S AND HATCHED IN THE SAME CONCENTRATION OR IN CONTROL WATER

Test	Test conditions[a]	H_2S concentration, mg/l				
Chronic 6_1						
series a		Control	0.0004	0.0012	0.0033	0.0063
	T—T	6.75	6.75	6.50	6.35	8.00
	T—C	6.75	6.50	6.50	6.35	6.50
series b		Control	0.0004	0.0011	0.0026	—[b]
	T—T	6.75	6.25	6.75	6.75	—
	T—C	6.75	6.25	—	6.75	—
series c		Control	0.0005	0.0012	0.0033	0.0068
	T—T	6.00	6.35	7.00	6.00	6.00
	T—C	6.00	6.25	—	6.00	6.00
Chronic 6_2						
series a		Control	0.0008	0.0014	0.0035	0.0080
	T—T	5.25	5.75	6.00	5.50	6.00
	T—C	5.25	5.25	5.50	5.75	—
series b		Control	0.0006	0.0012	0.0032	0.0054
	T—T	5.75	5.50	5.00	6.25	6.50
	T—C	5.75	5.00	5.00	5.25	6.00
series c		Control	0.0007	0.0014	0.0044	0.0074
	T—T	5.75	5.25	6.00	—	7.50
T—C	T—C	5.75	5.25	5.50	—	5.50

---

[a] T—T designates eggs laid in H_2S and incubated in the same concentration; T—C designates eggs laid in treatment and hatched in control water.

[b] Fish killed by low O_2.

Survival to Hatch and Length of Fry at Hatch

Percentage survival of eggs to hatch in controls and H_2S treatments varied from 80 to 92 in chronic tests 6_1 and 6_2 (Table 25). Eggs laid and hatched in the same H_2S concentration did not survive at different rates in different treatments nor when hatched in control water during chronic 6_1. In chronic 6_2 eggs laid in H_2S and incubated in control water in most cases had lower survival than those laid and incubated in the same H_2S treatment. Survival rate was not directly related to H_2S concentration during incubation.

The length of fry at hatch did not vary significantly with H_2S concentration during incubation in either chronic 6_1 or 6_2 (Table 26). Lengths and weights of adults at termination are given in Tables 27 and 28. No differences attributable to H_2S concentrations were noted.

COMPARISON OF FATHEAD POPULATIONS

Experimental Design

The possibility that different populations of fathead minnows might react differently to H_2S was tested by determining acute toxicity of H_2S to fish from four wild populations and two artificially reared fathead minnow populations (Table 29). The four wild populations of the same year class were collected at three different seasons (fall, winter and spring) from upper midwest lakes which varied widely in chemical characteristics (Table 30). The wild stocks were taken in the fall near the end of the first growing season, in January during the winter period of low dissolved oxygen concentration, cold temperature and maximum concentration of dissolved solids, and in June when the fish with least resistance to overwintering stresses had been eliminated and before spawning activity began to interfere with bioassay results. The wild populations were all considered to be natural with independent genetic characteristics.

Table 25. SURVIVAL TO HATCH OF FATHEAD MINNOW EGGS LAID IN VARIOUS CONCENTRATIONS OF H_2S AND INCUBATED IN THE SAME CONCENTRATIONS OR IN CONTROL WATER (expressed as percentage)

Test	Test conditions[a]	H_2S concentration, mg/l				
Chronic 6_1						
series a		Control	0.0004	0.0012	0.0033	0.0063
	T--T	80	47	88	67	73
	T--C	80	47	88	43	54
series b		Control	0.0004	0.0011	0.0026	--[b]
	T--T	66	43	72	81	--
	T--C	66	43	--	54	--
series c		Control	0.0005	0.0012	0.0033	0.0068
	T--T	59	58	91	73	90
	T--C	59	51	62	78	92
Chronic 6_2						
series a		Control	0.0008	0.0014	0.0035	0.0080
	T--T	70	74	57	82	69
	T--C	70	66	45	62	--
series b		Control	0.0006	0.0012	0.0032	0.0054
	T--T	68	76	27	68	82
	T--C	68	65	58	53	57
series c		Control	0.0007	0.0014	0.0044	0.0074
	T--T	73	68	62	--	66
	T--C	73	52	46	--	44

---

[a] T--T designates eggs laid in H_2S and incubated in same concentration; T--C designates eggs laid in treatment and hatched in control water.

[b] Fish killed by low O_2.

Table 26. LENGTH OF FATHEAD MINNOW FRY AT HATCH FROM EGGS LAID IN VARIOUS CONCENTRATIONS OF H_2S AND INCUBATED IN THE SAME CONCENTRATION OR IN CONTROL WATER (expressed in millimeters)

Test	Test conditions[a]	H_2S concentration, mg/1				
Chronic 6_1						
series a		Control	0.0004	0.0012	0.0033	0.0063
	T--T	5.5	5.4	5.5	5.2	5.4
	T--C	5.5	5.4	5.3	5.4	5.4
series b		Control	0.0004	0.0011	0.0026	--[b]
	T--T	5.2	5.3	5.3	5.4	--
	T--C	5.2	5.3	--	5.4	--
series c		Control	0.0005	0.0012	0.0033	0.0068
	T--T	5.4	5.2	5.2	5.4	5.4
	T--C	5.4	5.3	--	5.1	5.6
Chronic 6_2						
series a		Control	0.0008	0.0014	0.0035	0.0080
	T--T	5.4	5.5	5.5	5.6	5.4
	T--C	5.4	5.3	5.5	5.5	--
series b		Control	0.0006	0.0012	0.0032	0.0054
	T--T	5.6	5.5	5.3	5.5	5.9
	T--C	5.6	5.5	5.4	5.4	5.9
series c		Control	0.0007	0.0014	0.0044	0.0074
	T--T	5.4	5.6	5.5	--	5.5
	T--C	5.4	5.6	5.6	--	5.4

---

[a] T--T designates fry from eggs laid and incubated in same treatment; T--C, fry from eggs laid in H_2S and incubated in control water.

[b] Fish killed by low O_2.

Table 27. LENGTH OF FATHEAD MINNOW ADULTS AT TERMINATION OF CHRONIC TESTS 6_1 AND 6_2
(millimeters total length)

Test	H_2S concentration, mg/l				
Chronic 6_1					
series a	Control	0.0004	0.0012	0.0033	0.0063
$\bar{x}$ length males[a]	70.8	72.9	73.2	73.6	74.2
$\bar{x}$ length females	59.8	54.6	56.8	55.5	56.5
$\bar{x}$ length M & F	65.2	63.8	70.0	68.4	63.6
series b	Control	0.0004	0.0011	0.0026	—[b]
$\bar{x}$ length males	78.2	72.7	73.5	71.5	—
$\bar{x}$ length females	57.0	57.2	58.2	54.9	—
$\bar{x}$ length M & F	63.1	63.9	67.8	58.2	—
series c	Control	0.0005	0.0012	0.0033	0.0068
$\bar{x}$ length males	76.0	69.8	73.3	78.0	70.5
$\bar{x}$ length females	54.8	55.2	55.2	55.5	—
$\bar{x}$ length M & F	66.6	61.7	66.1	66.8	70.5
Chronic 6_2					
series a	Control	0.0008	0.0014	0.0035	0.0080
$\bar{x}$ length males	72.2	71.7	70.8	66.4	—
$\bar{x}$ length females	59.9	53.5	56.5	54.8	—
$\bar{x}$ length M & F	63.6	64.4	62.2	61.8	—
series b	Control	0.0006	0.0012	0.0032	0.0054
$\bar{x}$ length males	72.2	71.8	70.0	67.0	72.6
$\bar{x}$ length females	57.3	51.8	54.8	54.9	52.8
$\bar{x}$ length M & F	63.3	67.3	64.9	59.4	67.6

Table 27 continued. LENGTH OF FATHEAD MINNOW ADULTS AT TERMINATION OF CHRONIC TESTS 6_1 AND 6_2

(millimeters total length)

Test	H_2S concentration, mg/l				
Chronic 6_2					
series c	Control	0.0007	0.0014	0.0044	0.0074
$\bar{x}$ length males	71.5	71.0	72.8	—	69.8
$\bar{x}$ length females	56.9	55.4	54.2	—	54.7
$\bar{x}$ length M & F	58.5	58.8	61.2	—	62.2

[a] All fish lengths reported as total length.
[b] Fish killed by low O_2.

Table 28. WEIGHT OF FATHEAD MINNOW ADULTS AT TERMINATION OF CHRONIC TEST 6_1

(grams wet weight)

Test	H_2S concentration, mg/l				
Series a	Control	0.0004	0.0012	0.0033	0.0063
Mean weight males	4.054	4.145	4.058	4.221	4.552
Mean weight females	2.155	1.370	1.612	1.522	1.529
Mean weight M & F	3.105	2.757	3.569	3.450	2.738
Series b	Control	0.0004	0.0011	0.0026	--[a]
Mean weight males	5.184	5.201	4.293	4.257	--
Mean weight females	1.574	1.655	1.788	1.388	--
Mean weight M & F	2.605	3.175	3.353	1.962	--
Series c	Control	0.0005	0.0012	0.0033	0.0068
Mean weight males	4.760	3.758	4.479	5.774	3.980
Mean weight females	1.495	1.466	1.452	1.181	--
Mean weight M & F	3.303	2.485	3.268	3.478	3.980

[a] Fish killed by low O_2.

The artificially reared stocks came from fish reared in the National Water Quality Laboratory at Duluth, Minnesota and from fish reared in the fishery laboratories at the University of Minnesota. Both stocks originated from ponds at the Newtown Laboratory and presumably from the same gene pool. Prior to acute toxicity tests, minnows collected in the fall and spring were acclimated to 20 C and from winter collections to 14 C, the test temperatures, for at least 10 days prior to the start of tests. Prophylactic treatments with 20 mg/liter neomycin were carried out in the acclimation tanks immediately after collection of each group. Acute bioassays were conducted in the diluter systems previously described for acute fathead minnow tests with juveniles. Four treatments and one control were used in each test. The pH was held at 7.7 and dissolved oxygen at 5-6 mg/liter. Sulfide concentrations were measured in the center of each test chamber twice each day and other determinations were made once daily. Bioassays were continued until 48 hr elapsed without additional mortality. All tests were replicated three times.

Acute Toxicity

Fall collections from the four lakes had mean threshold LC50's at 20 C from 0.023 mg/liter in Wakefield Lake (Table 31) to 0.029 mg/liter H_2S in Hay Lake (Table 32). The fall Wakefield Lake sample had an abnormally high mortality rate during acclimation, which may account for its relatively low resistance to H_2S (Table 34). Winter collections could be made only from Harrier Lake (Table 33) and Hay Lake. These fish showed greater resistance than fish collected in the fall with LTC at 14 C of 0.038 mg/liter in Harrier Lake and 0.032 mg/liter H_2S in Hay Lake (Table 34). This discrepancy is consistent with the effect of test temperature on H_2S toxicity shown in Table 4. There were no winter collections from Porter Lake. Samples of wild fish collected in the spring of 1973 were all in healthy condition. Mean LTC's at 20 C varied between lakes from 0.028 to 0.030 mg/liter H_2S.

The two groups of cultured fish varied little between themselves with

Table 29. SOURCE OF FATHEAD MINNOWS USED FOR
POPULATION COMPARISON TESTS

Test No.	Source	Date	Temperature, C	Collection method
A1F	Harrier	9/21/72	11.5	Seine
A2F	T137N R71W	"	"	"
A3F	Kidder Cty, ND	"	"	"
A4W	Harrier	1/7/73	Ice cover	Trap
A5W	"	"	"	"
A6W	"	"	"	"
A7S	Harrier	5/30/73	16.0	Seine
A8S	"	"	"	"
A9S	"	"	"	"
B1F	Wakefield	10/16/72	9.5	Seine
B2F	T29N R22W	"	"	"
B3F	Ramsey Cty, MN	"	"	"
B4S	Wakefield	5/23/73	18.5	Seine
B5S	"	"	"	"
B6S	"	"	"	"
C1F	Hay	10/25/72	6.0	Trap
C2F	T32N R20W	"	"	"
C3F	Washington Cty, MN	"	"	"
C4W	Hay	2/2/73	Ice cover	Trap
C5W	"	"	"	"
C6W	"	"	"	"

Table 29 continued. SOURCE OF FATHEAD MINNOWS USED FOR POPULATION COMPARISON TESTS

Test No.	Source	Date	Temperature, C	Collection method
C7S	Hay	5/31/73	22.0	Seine
C8S	"	"	"	"
C9S	"	"	"	"
C10S	"	"	"	"
D1F	Porter	10/26/72	6.5	Seine
D2F	T118N R30W	"	"	"
D3F	Meeker Cty, MN	"	"	"
D4S	Porter	5/22/73	20.0	Seine
D5S	"	"	"	"
D6S	"	"	"	"
E1S	U of M	6/27/73	25.0	-
E2S	"	"	"	-
E3S	"	"	"	-
F1S	Newtown, Ohio	8/15/73	26.0[a]	-
F2S	"	"	"	-
G1S	Duluth NWQL	8/31/73	24.0	-
G2S	"	"	"	-
G3S	"	"	"	-

[a] On receipt by air freight.

Table 30. LAKE WATER CHEMISTRY[a]
(concentrations in mg/l)

Compound or element	Hay	Wakefield	Porter	Harrier
Total hardness as $CaCO_3$	9	40	190	250
Total alkalinity as $CaCO_3$	14	46	200	450
Total phosphorus	0.10	0.08	0.11	0.24
Ammonia nitrogen	0.05	0.20	0.17	0.05
Organic nitrogen	2.4	1.4	1.9	1.1
Sulfate	5.1	6.4	8.5	430
Chloride	3	48[b]	8	22
Manganese	0.05	0.01	0.01	0.07
Iron	0.27	0.06	0.06	0.35
Calcium as $CaCO_3$	7	32	93	30
Sodium	3	48[b]	8	310
Potassium	2	27[b]	6	46
Magnesium as $CaCO_3$	2	8	97	220
Fluoride	0.1	0.1	0.1	0.1
Specific conductivity µmho/cm @ 25 C	27	200	280	1500

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[a] Water samples taken with fall collection of fish; analyses by Minn. Dept. of Health.

[b] High concentrations probably due to wintertime salting of residential streets near the lake.

Table 31. ACUTE TEST CONDITIONS AND LC50 VALUES FOR FATHEAD MINNOWS IN POPULATION COMPARISON (WAKEFIELD LAKE)

Test No.	Days from collection to start of test	Mean length, mm	Mean test conditions: Temp., C	Mean test conditions: O_2, mg/1	LC50, mg/1 H_2S: 24 hr	48 hr	72 hr	96 hr	LTC (days)
B1F[a]	16	28.5	20.1	5.5	–	0.033	0.025	0.031	0.024 (9)
B2F	29	28.5	20.1	5.8	–	–	–	0.024	0.024 (6)
B3F	38	33.0	20.2	5.6	–	0.032	0.023	0.022	0.022 (7)
									$\bar{x}$ = 0.023
B4S[b]	30	43.5	20.1	5.5	0.037	0.028	0.026	0.026	0.026 (6)
B5S	37	47.0	19.9	5.5	0.036	0.035	0.031	0.029	0.029 (6)
B6S	37	49.5	20.0	5.6	–	0.032	0.030	0.030	0.030 (6)
									$\bar{x}$ = 0.028

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[a] Fall collections.
[b] Spring collections.

Table 32. ACUTE TEST CONDITIONS AND LC50 VALUES FOR FATHEAD MINNOWS IN POPULATION COMPARISON (HAY LAKE)

Test No.	Days from collection to start of test	Mean length, mm	Mean test conditions Temp., C	Mean test conditions O_2, mg/l	LC50, mg/l H_2S 24 hr	48 hr	72 hr	96 hr	LTC (days)
C1F[a]	39	37.0	19.9	5.7	–	0.037	0.032	0.032	0.032 (5)
C2F	64	38.5	20.0	5.5	–	–	0.033	0.030	0.029 (7)
C3F	80	41.5	20.0	5.6	–	0.040	0.035	0.028	0.027 (7)
									$\bar{x}$ = 0.029
C4W[b]	57	50.0	13.9	5.9	0.065	0.052	0.043	0.039	–
C5W	75	51.5	14.0	5.8	–	–	–	0.041	0.032 (11)
C6W	75	50.5	13.8	5.9	–	–	0.045	0.040	0.032 (11)
									$\bar{x}$ = 0.032
C7S[c]	46	46.5	20.0	5.7	–	0.038	0.035	0.032	0.032 (6)
C8S	55	50.5	20.0	5.5	–	0.041	0.036	0.033	0.029 (7)
C9S	55	51.5	19.9	5.7	–	0.042	0.036	0.031	0.029 (7)
									$\bar{x}$ = 0.030

[a] Fall collection (CF).
[b] Winter collection (CW).
[c] Spring collection (CS).

Table 33. ACUTE TEST CONDITIONS AND LC50 VALUES FOR FATHEAD MINNOWS IN POPULATION COMPARISON (HARRIER LAKE)

			Mean test conditions		LC50, mg/1 H_2S				
Test No.	Days from collection to start of test	Mean length, mm	Temp., C	O_2, mg/1	24 hr	48 hr	72 hr	96 hr	LTC (days)
A1F[a]	41	33.0	19.9	5.6	-	-	0.033	0.028	0.026 (9)
A2F	54	32.0	20.1	5.6	-	0.056	0.027	0.027	0.027 (6)
A3F	63	36.0	20.0	5.4	-	0.031	0.029	0.026	0.026 (7)
									$\bar{x}$ = 0.026
A4W[b]	44	45.0	14.0	6.1	-	-	0.061	0.059	0.037 (13)
A5W	44	43.5	13.9	6.4	-	0.071	0.066	0.059	0.042 (13)
A6W	82	47.5	13.9	6.3	-	0.045	0.043	0.043	0.036 (12)
									$\bar{x}$ = 0.038
A7S[c]	40	49.0	20.1	5.4	0.039	0.033	0.030	0.029	0.029 (6)
A8S	40	45.5	20.0	5.6	0.040	0.032	0.032	0.030	0.030 (6)
A9S	47	50.0	20.0	5.7	0.039	0.035	0.031	0.031	0.031 (6)
									$\bar{x}$ = 0.030

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[a] Fall collection (AF).

[b] Winter collection (AW).

[c] Spring collection (AS).

Table 34. ACUTE TEST CONDITIONS AND LC50 VALUES FOR FATHEAD MINNOWS IN POPULATION COMPARISON (PORTER LAKE)

Test No.	Days from collection to start of test	Mean length, mm	Mean test conditions Temp., C	Mean test conditions O_2, mg/1	LC50, mg/1 H_2S 24 hr	LC50, mg/1 H_2S 48 hr	LC50, mg/1 H_2S 72 hr	LC50, mg/1 H_2S 96 hr	LC50, mg/1 H_2S LTC (days)
D1F[a]	37	35.0	20.1	5.7	-	0.047	0.032	0.028	0.027 (6)
D2F	62	39.0	20.0	5.3	-	-	0.033	0.028	0.028 (6)
D3F	78	42.5	20.1	5.7	-	0.040	0.030	0.028	0.026 (8)
									$\bar{x}$ = 0.027
D4S[b]	15	46.0	20.1	5.6	0.041	0.036	0.032	0.031	0.030 (7)
D5S	15	47.5	19.9	5.7	-	0.038	0.035	0.031	0.029 (7)
D6S	30	43.5	20.0	5.6	0.040	0.034	0.032	0.031	0.031 (6)
									$\bar{x}$ = 0.030

[a] Fall collections.
[b] Spring collections.

mean LTC's ranging from 0.018 to 0.019 mg/liter H_2S (Table 35). These acutely toxic levels were substantially lower, however, than the LTC levels of the wild populations (Table 36). Reasons for this discrepancy in resistance appear to be related either to continued laboratory culture in which less resistant fish were held in the gene pool or genetic differences in the original Newtown stock. In work reported above where wild eggs were brought into the laboratory and hatched and reared to juveniles under laboratory conditions, the fish had essentially the same resistance as wild juveniles from the same stock. If disease in the fall Wakefield sample is taken into account, the results described here indicate that various wild stocks, in the limited geographic area covered by these experiments, do not have significantly different resistance to H_2S. Temperature variations and the season when fish are collected have more influence than difference in genetic stock.

SUMMARY

Acute tests of H_2S with fathead minnows show that 96-hr LC50 ranged from 0.0280 mg/liter at 25 C to 0.5800 mg/liter at 4 C. Wild stock from various lakes in Minnesota and North Dakota varied in response with season and temperature but not with geographic location. Laboratory-reared fish from Minnesota stock did not vary from wild stock but laboratory-reared fish from Duluth (Newtown) culture stock were more than twice as sensitive at 20 C as local wild stock (96-hr LC50 of 0.0162 and 0.0367 mg/liter H_2S respectively).

Chronic tests (time periods greater than that required for LTC) with wild stock showed survival was affected adversely at 0.0078 mg/liter H_2S and higher. Growth of wild stock was inhibited at 0.0080-0.0093 mg/liter and higher and Duluth stock at 0.0052 mg/liter H_2S and higher when tests were started with juveniles. When all life history stages were exposed, growth of Duluth stock was reduced at 0.0033 mg/liter H_2S and higher. Fecundity in chronic exposure from egg to spawning adult did not appear to be related to H_2S concentration up to 0.0070-0.0080 mg/liter although delay in first spawning occurred at the higher test levels.

Table 35. ACUTE TEST CONDITIONS AND LC50 VALUES FOR FATHEAD MINNOWS IN POPULATION COMPARISON (DULUTH-NEWTOWN STOCK)

	Days from collection to start	Mean	Mean test conditions		LC50, mg/1 H_2S				
Test No.[a]	of test	length, mm	Temp., C	O_2, mg/1	24 hr	48 hr	72 hr	96 hr	LTC (days)
E1S	51	40.5	20.0	5.4	0.028	0.020	0.018	0.017	0.017 (6)
E2S	51	41.0	19.9	5.8	0.030	0.026	0.022	0.019	0.018 (6)
E3S	58	42.0	20.0	5.5	-	-	0.022	0.021	0.020 (8)
									$\bar{x}$ = 0.018
F1S	32	39.5	20.0	5.4	0.032	0.020	0.018	0.017	0.017 (6)
F2S	32	37.5	20.0	5.7	-	-	-	0.022	0.021 (6)
									$\bar{x}$ = 0.019
G1S	10	41.0	20.1	5.5	-	0.024	0.019	0.018	0.017 (7)
G2S	10	41.5	20.0	5.8	-	0.025	0.023	0.022	0.020 (7)
G3S	21	46.5	20.0	5.6	-	0.029	0.021	0.020	0.019 (7)
									$\bar{x}$ = 0.019

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[a] E series - University of Minnesota stock; F series - Newtown stock; G series - Duluth stock.

Table 36. LTC VALUES OF FATHEAD MINNOWS FROM SEVEN POPULATIONS

Population	Test temperature, C	LTC (days), mg/liter H_2S
Harrier Lake		
Fall	20	0.026 (6-9)
Winter	14	0.038 (12-13)
Spring	20	0.030 (6)
Wakefield Lake		
Fall	20	0.023 (6-9)
Spring	20	0.028 (6)
Hay Lake		
Fall	20	0.029 (57)
Winter	14	0.032 (11)
Spring	20	0.030 (6-7)
Porter Lake		
Fall	20	0.027 (6-8)
Spring	20	0.030 (6-7)
University of Minnesota stock	20	0.018 (6-8)
Newtown stock	20	0.019 (7)
Duluth stock	20	0.018 (7)

SECTION VI

GOLDFISH

(Carassius auratus (Linnaeus))

The response of goldfish to H_2S was tested by (1) a series of acute bioassays at various temperatures on a strain of goldfish from a commercial hatchery and a second strain from a federal hatchery, (2) a series of chronic bioassays, and (3) a series of acute bioassays to determine factors affecting bioassay variability.

ACUTE TESTS

Experimental Design

Acute bioassays of H_2S were conducted on the various life history stages of the goldfish. A total of one test with eggs, one with newly hatched fry, and 112 with juveniles were run. Of the juvenile tests, 29 were to determine temperature effects, 20 oxygen effects, and 63 bioassay method effects.

The egg bioassays were conducted using the modified proportional diluter previously described. A control and four toxicant concentrations were dispensed from the diluter into a two-chambered glass container. The incoming water and sodium sulfide stock solution flowed into a 20 x 20 x 10 cm deep mixing chamber and then through a perforated glass tube into a 10 x 10 x 5 cm deep test chamber. The eggs were not moved by the flow of water. Eggs were artificially spawned from a stock of adult goldfish obtained from Ozark Fisheries, Inc., Stoutland, Missouri.

Adults were raised from 11 to 20 C in 2 days to initiate spawning and eggs from four females were hand stripped. Eggs were fertilized with milt from excised testes of four males. They were exposed to H_2S from 4 hr after fertilization through hatching.

One 96-hr bioassay with fry was conducted using the modified proportional diluter. Fry test chambers were 7.5 x 7.5 x 10 cm deep with three glass sides and bottom and one Nitex (nylon) screened side. The water flowed from the mixing chamber into the fry chamber, then out through the screen into an aquarium in which the chamber was immersed. Fry were not fed during the bioassay and were not ready to accept food until the last day of the test.

Acute bioassays with juveniles for determination of the effect of temperature on H_2S toxicity were conducted in two identical continuous-flow units in which gaseous H_2S was mixed with well water in an apparatus described by Colby and Smith[1] with modifications by Adelman and Smith[2]. Each unit included six 25-liter glass test chambers (one control and five treatments), measuring 40 x 25 x 28 cm deep. With a flow rate of 280 ml/min, 90% replacement of water occurred in approximately 1-1/4 hr.

Two stocks of goldfish from Ozark Fisheries, Inc. and three stocks from the Federal Hatchery at Lake Mills, Wisconsin were tested for temperature effects during a 16-month period. Variation in fish age in different bioassays was from approximately 4 months to 2-1/2 years, and the range in mean weights was from 1.40 to 14.63 g. Within any bioassay the age, weight, and stock of fish was the same.

Fish from both sources were treated with 0.86 mg/liter methylene blue for 2 days with an additional 0.86 mg/liter added on the third day to control skin fluke (*Gyrodactylus* sp.) infections. The fish were held in the solution for 5 days. Fish held in the laboratory were retreated periodically when the incidence of flukes increased. Fish were not used for bioassay until at least 2 weeks after treatment.

One week prior to a bioassay 65 fish from each stock to be tested were removed from holding tanks where temperatures ranged from 10 to 13 C and placed in a 38-liter acclimating aquarium and then transferred to test chambers. Oxygen concentration was maintained near saturation and water flow was continuous. Temperatures were raised or lowered from that of the holding room to the desired bioassay temperature at the rate of 4 C per day, and held at test temperature at least 3 days prior to the start of the test. Fish were fed Oregon moist pelleted trout food until 1 day prior to the bioassay but not during test. In each bioassay 10 fish were placed in each chamber after random stratified assortment. Hydrogen sulfide was then raised to the desired concentration within a period of 3 hr. Mortality was recorded at 24-hr intervals.

The two units used for temperature series were also used for bioassays with different oxygen levels. The test chambers for oxygen tests were acrylic plastic egg-hatching jars (depth 45 cm, diameter 14.5 cm, volume of water 6.1 liters). Water replacement was 90% in approximately 50 min.

Five stocks of goldfish from Ozark Fisheries, Inc., all hatched during May and June, 1970, were used for bioassays to determine effects of oxygen. Age of fish ranged from 6 to 17 months and mean weights from 3.95 to 6.06 g. During the holding period skin flukes were treated for 1 hr on 2 consecutive days with 0.25 mg/liter formalin. Bioassays were conducted not less than 10 days after treatment.

Tests were conducted in pairs with a different oxygen concentration in each of the two. One week before the start of a test, 100 fish were divided between two 38-liter aquaria for acclimation. Oxygen was held at saturation when temperature acclimation only was required. Acclimation to specified oxygen concentrations was achieved by continuous withdrawal of water from a reaeration system described by Brungs.[8] Oxygen concentrations were adjusted to the desired level at intervals over 24 hr and temperature was raised from 10 to 11 C to 17.5 C in 2 days for both groups. After desired levels were attained, fish were held for 1 week before start of test.

At the start of a pair of bioassays fish were assigned in a stratified random manner, eight per chamber, to five treatments and one control in which the desired oxygen and H_2S concentrations had previously been set. Transfer was done as rapidly as manipulation permitted.

Sixty-three bioassays were conducted to determine factors affecting bioassay variability. In this series three proportional diluters were used to deliver water to three series of four treatments and one control. Test chambers measured 50 x 25 x 20 cm and contained 20 liters. With a flow rate of 445 ml/min, 90% replacement of water occurred in approximately 1-3/4 hr.

This experiment was conducted in a 3 x 3 factorial design with three temperatures (14, 20, and 26 C), three acclimation times (1, 3, and 5 weeks), and seven blocks (all fish were from the same source but seven different batches). Approximately every 8 weeks a new shipment of goldfish was obtained from Ozark Fisheries. Six of these stocks were hatched in May and June, 1970 and one in May, 1971. Their age at time of bioassay ranged from 6 to 15 months, and mean weights ranged from 3.17 to 5.70 g. On arrival in the laboratory the temperature was lowered from that of the shipment water (15-23 C) to the holding tank temperature (10-13 C) within 24 hr, and on the second and third day after arrival the formalin treatment described above was applied. Three days later 500 of the fish were divided into three groups, and each group was placed in a 400-liter acclimation tank. The temperature was raised to the desired levels of 14, 20, and 26 C at the rate of 5 C per day.

After 1, 3, and 5 weeks from the start of acclimation approximately one-third of the stock in each tank was removed for an 11-day bioassay of H_2S at the acclimation temperature. The fish from each group were placed in the five chambers of the appropriate diluter in a stratified random manner. The desired H_2S concentrations had been attained in these chambers and fish were not fed during the entire bioassay. Hydrogen sulfide, pH, and temperature were measured once per day and dissolved oxygen once per week.

Acute Toxicity

Eggs and Fry--In the egg bioassay the mean pH reading was 7.62 (range 7.55-7.73); mean temperature 22.1 C (range 22.0-22.2); and mean dissolved oxygen 8.72 mg/liter (range 8.25-9.25).

The percent survival at hatch decreased in the two highest H_2S concentrations (Table 37) with 90% loss at 0.0291 mg/liter H_2S. The LC50 at hatching computed from percentage of all survivals corrected for mortality in controls (Abbott[9]) was 0.022 mg/liter H_2S and for normal fry only 0.020 mg/liter. In the two highest concentrations where egg mortality occurred, a greater percentage of malformed fry were observed (Table 37), with 62% at 0.0291 mg/liter H_2S.

In the fry bioassay the mean pH was 7.66 (range 7.52-8.08); mean temperature 21.6 C (range 19.8-22.2); and mean oxygen was 8.29 mg/liter (range 8.15-8.50).

Fry survival was considerably less in the two highest H_2S concentrations (Table 38) with complete kill at 0.0485 mg/liter. The 96-hr LC50 was 0.025 mg/liter. The length of fry at the termination of the bioassay was less in all H_2S treatments and decreased with increasing H_2S. A t-test indicated that the length of fry in the lowest H_2S concentration was significantly different than the control ($t_{.01,49} = 2.3448$).

Juveniles--Temperature effects--In the 29 bioassays to determine the effects of temperature on H_2S toxicity the mean pH reading was 7.80

Table 37. PERCENTAGE SURVIVAL OF GOLDFISH EGGS AND PERCENTAGE MALFORMED FRY IN VARIOUS H_2S CONCENTRATIONS

H_2S (mg/l) - mean	Control	0.0052	0.0097	0.0169	0.0291
range	-	(0.0044-0.0066)	(0.0075-0.0112)	(0.0124-0.0197)	(0.0241-0.0357)
No. surviving fry	68	75	76	58	8
Survival (%)	85	94	95	72	10
Malformed fry (%)[a]	0	1	0	14	62

[a] Percentage of total fry.

Table 38. PERCENTAGE SURVIVAL OF GOLDFISH FRY AND MEAN LENGTH OF FRY AT END OF EXPOSURE TO VARIOUS H_2S CONCENTRATIONS

H_2S (mg/l) - mean	Control	0.0140	0.0207	0.0271	0.0485
range	-	(0.0056-0.0187)	(0.0092-0.0282)	(0.0131-0.0350)	(0.0213-0.0593)
No. surviving fry[a]	50	47	47	13	0
Survival (%)	100	94	94	26	0
Mean length (mm)	6.83	6.54	6.53	5.53	-

[a] 50 fry at start of test in each treatment.

(range 7.63-7.99; standard deviation, 0.097), the mean dissolved oxygen was 5.8 mg/liter (range 4.8-7.1; standard deviation 0.68), and mean total alkalinity was 237 mg/liter $CaCO_3$ (range 225-262; standard deviation, 7.3).

The 96-hr LC50 ranged from 0.556 mg/liter at 6.7 C to 0.044 mg/liter at 25 C (Table 39). The log of the LC50 increases proportionally to a decrease in the log of temperature (Figure 2). The linear regression is described by the equation:

$$\log \hat{y} = 4.2325 - 1.8527 \log x \qquad (1)$$

where $\hat{y}$ = 96-hr LC50

x = temperature.

The regression is highly significant ($F_{1,25} = 254.86$, $p < .01$), and 91% of the variation in LC50 is attributable to temperature (Steel and Torrie[10]). The regression line for the goldfish is compared with that for fathead minnows and with that for the uniform stocks of goldfish in the tests of bioassay variability reported below (Figure 3). The increase in toxicity of H_2S with increasing temperatures is similar for the three groups with all goldfish more tolerant than the fathead minnows and the uniform stock of goldfish more tolerant then the mixed stocks. Since the three points defining the regression line for the uniform group are the means of 21 bioassays (described in the following section), they are more precise than any single point defining the regression for the mixed stock where a maximum of six bioassays was conducted at an individual temperature. The regression line for the uniform stock indicates that at least with this group the effect of temperature is slightly curvilinear on a logarithmic plot.

Table 39. LC50 OF H_2S TO GOLDFISH AT DIFFERENT TEMPERATURES

Test[a]	Temperature, C	Mean length of fish, mm	Mean 96-hr LC50, mg/l H_2S
LM-10	25.0	37.5	0.044
LM-17	24.9	60.3	0.065
O-9	24.8	28.8	0.037
O-16	24.6	40.9	0.049
O-7	24.2	25.7	0.034
LM-8	24.0	39.1	0.050
O-4	23.1	1.77	0.051
O-2	23.0	14.0	0.057
LM-3	23.0	27.8	0.063
O-5	23.0	22.8	0.050
LM-6	23.0	27.6	0.054
O-18	22.9	51.6	0.050
O-1	20.3	17.5	0.048
O-20	20.0	57.8	0.061
LM-19	19.8	66.0	0.057
LM-14	17.0	47.2	0.094
O-15	17.0	33.0	0.053
LM-23	15.1	88.7	0.118
LM-27	15.0	144.0	0.110
LM-24	13.9	109.8	0.193
O-12	12.4	35.1	0.175
LM-13	12.3	50.5	0.276
O-11	12.2	34.9	0.133
O-25	10.2	116.1	0.271
LM-26	10.2	134.4	0.286
LM-28	8.8	146.3	0.241
O-21	6.7	69.9	0.556
LM-22	6.7	75.2	0.375

[a] O - Ozark stock; LM - Lake Mills stock

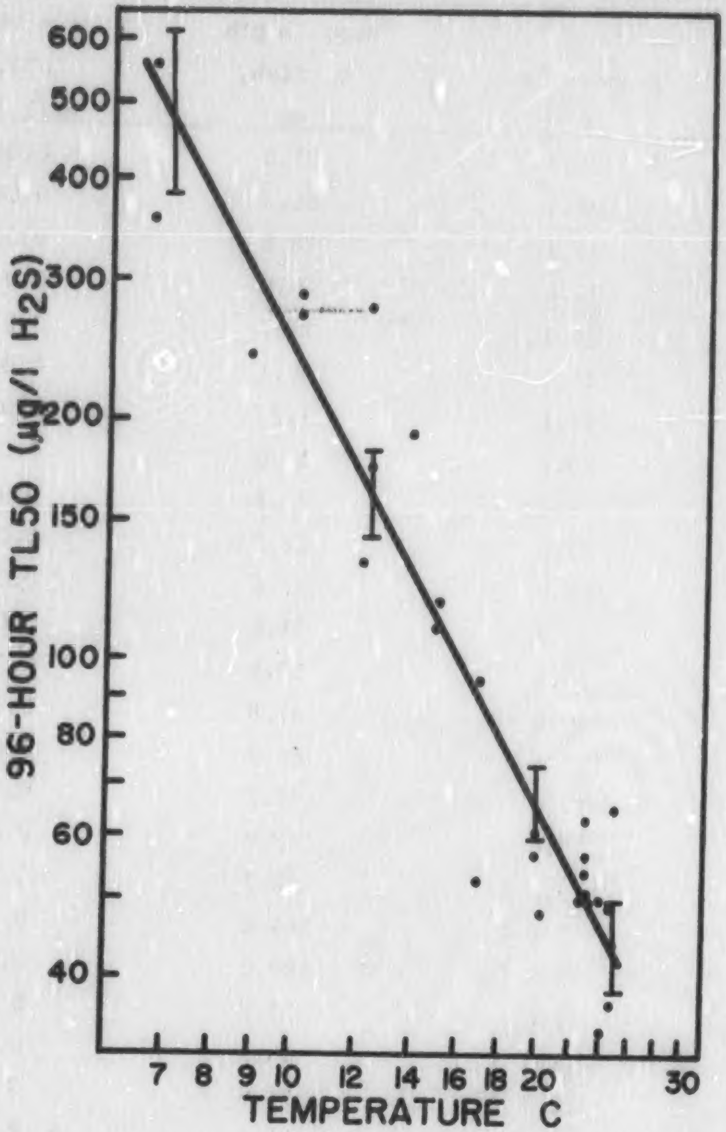

Figure 2. The effect of temperature on the 96-hr LC50 (TL50) of H_2S to goldfish with 95% confidence limits.

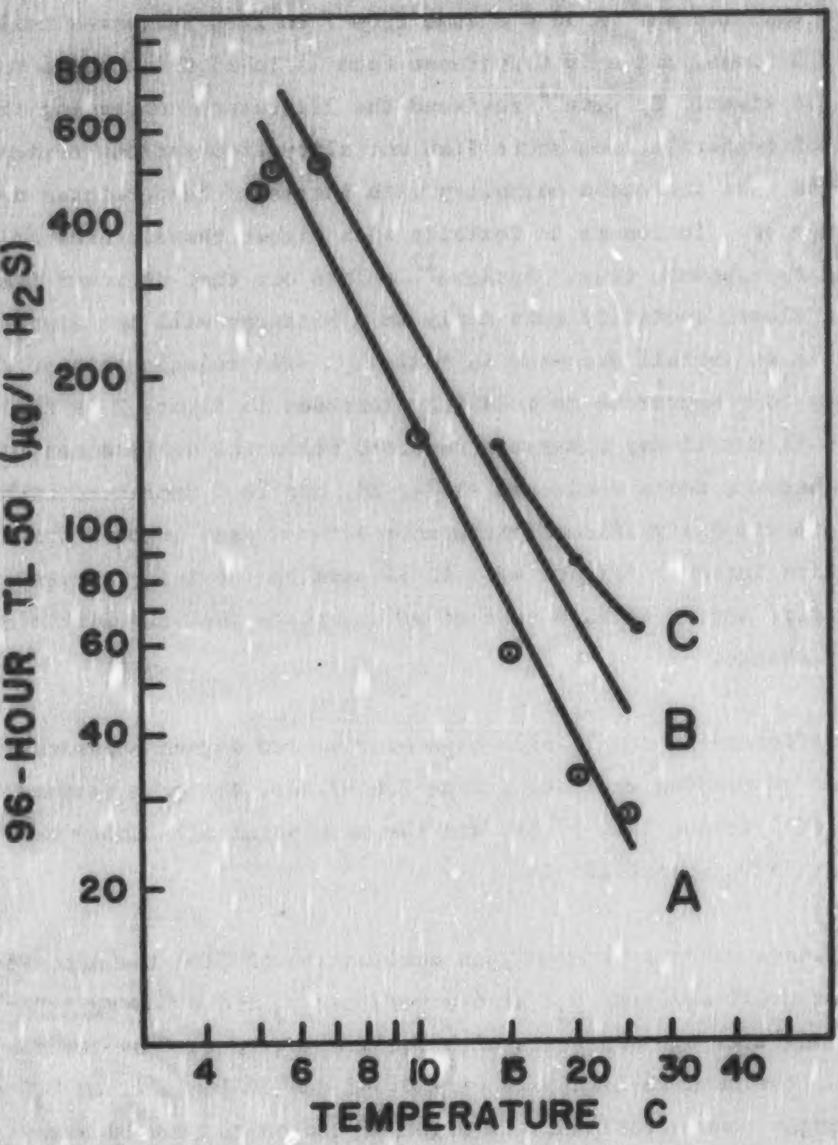

Figure 3. The effect of temperature on the 96-hr LC50 (TL50) of H_2S to three groups of fish: (A) fathead minnow, (B) goldfish from bioassays on temperature effects, (C) goldfish from bioassays for determination of variability in LC50 caused by various factors.

Since the relationship of the 96-hr LC50 to temperature is logarithmic, increases in tolerance at the colder temperatures are larger than at higher temperatures. A 10 C change from 7 to 17 C increases toxicity about 5.5 times, but a 10 C increase from 15 to 25 C increases toxicity about 2.6 times. Sprague[11] reviewed the literature concerning the effect of temperature on acute fish mortality from various toxicants and noted that increased mortality with increased temperatures was quite common. Increases in toxicity with higher temperatures are related to exposure time. Sprague[11] points out that at lower temperatures a slower mortality rate early in a bioassay will not always result in an overall decrease in toxicity. The relationship of H_2S toxicity to temperature in goldfish expressed in Figure 2 is for 96-hr tests. In the 11-day bioassays reported below the differences in LC50 values between tests conducted at 14, 20, and 26 C decreased with time, but there was a significant difference between each temperature at every time interval (Figure 4). At 11 days the toxicity curves at each temperature appear to have reached an asymptote implying little subsequent change.

Oxygen effects--In the 21 bioassays with varied oxygen concentrations the mean pH reading was 7.69 (range 7.64-7.80), the mean temperature was 17.46 C (range 17.0-17.8), and the mean total alkalinity was 229 mg/liter $CaCO_3$ (range 205-245).

In bioassays without prior oxygen acclimation of fish the mean 96-hr LC50 was 0.071 mg/liter H_2S at 6.0 mg/liter O_2 and 0.053 mg/liter H_2S at 1.5 mg/liter O_2. In tests with acclimation to test oxygen concentration, the mean 96-hr LC50's were 0.062 and 0.048 mg/liter H_2S at the same oxygen concentrations. In eight of ten pairs, the bioassay conducted at the lower O_2 concentration resulted in a lower 96-hr LC50 (Tabl 40). Shelford[12], Colby and Smith[1], and Adelman and Smith[2] found this same effect in other species although the latter authors found no effect of oxygen differences on toxicity of H_2S to northern pike eggs.

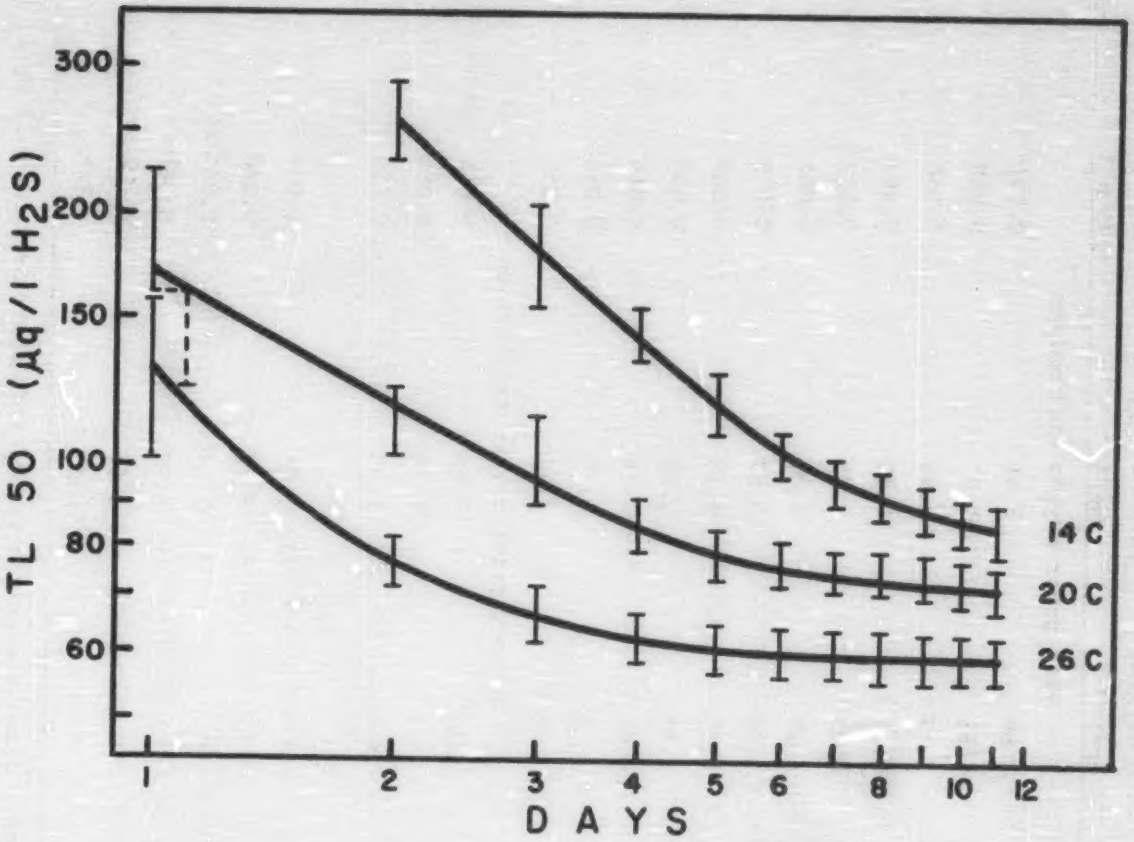

Figure 4. Changes in LC50 (TL50) values during 11 days of exposure to H_2S at three temperatures with 95% confidence limits.

Table 40. EFFECT OF OXYGEN ON H_2S TOXICITY TO GOLDFISH IN PAIRED BIOASSAYS

Bioassay No.	Mean acclimation oxygen, mg/1	Mean oxygen in bioassay, mg/1	96-hr LC50, mg/1 H_2S
	With prior oxygen acclimation		
1A	2.88	3.41	0.051
1B	4.83	4.97	0.058
2A	2.13	1.96	0.058
2B	6.07	6.13	0.063
3A	1.25	1.05	0.070
3B	4.16	4.36	0.054
4A	3.08	3.07	0.055
4B	6.55	6.13	0.066
10A	1.39	1.00	0.095
10B	4.06	4.16	0.049
12A	1.50	1.40	0.046
12B	2.84	2.29	0.050
	Without prior oxygen acclimation		
5A	Sat.[a]	1.18	0.044
5B	"	4.28	0.053
6A	"	1.95	0.055
6B[b]	-	-	-
7A	"	1.81	0.049
7B	"	5.72	0.080
8A	"	2.81	0.066
8B	"	6.31	0.069
9A	"	1.08	0.060
9B	"	4.83	0.065

[a] Saturation. Dissolved oxygen varied from approximately 9-11 mg/1.
[b] Bioassay not completed due to apparatus failure.

In two bioassays (10A, 3A, Table 40) where the fish were acclimated to the O_2 concentration of the bioassay, the 96-hr LC50's were higher than all but one in the unacclimated series. These two values were eliminated because they deviated considerably from the other values and since they occur at very low O_2 concentrations they may be indicative of a separate phenomenon. With these two values eliminated, the relationship of the 96-hr LC50 to the O_2 concentration forms the significant linear regression $F_{1,8} = 9.78$, $p<.05$ (Steel and Torrie[10]):

$$\hat{y} = .0443 + 2.83\,x \qquad (2)$$

where $\hat{y}$ = 96-hr LC50
x = oxygen concentration

(Figure 5). The linear regression from bioassays without oxygen acclimation is also significant ($F_{1,7} = 8.09$, $p<.05$):

$$\hat{y} = .0465 + 4.08\,x \qquad (3)$$

where $\hat{y}$ and x are the same as above (Figure 5). There is a considerable variation among points around both regression lines since in the former only 55% and in the latter 54% of the variation in the 96-hr LC50 is attributable to O_2 concentration. The rate of increase in H_2S toxicity with decreasing oxygen is the same with or without prior acclimation as was shown by an analysis of covariance ($F_{1,15} = .52$, n.s. at .05) (Snedecor and Cochran[13]).

The analysis also indicated that the elevation of the regression line for bioassays without acclimation was significantly higher than that for bioassays with acclimation ($F_{1,16} = 5.23$, $p<.05$). Acclimation to lower oxygen concentration prior to testing does not increase the resistance of goldfish to acute H_2S toxicity but makes them more sensitive.

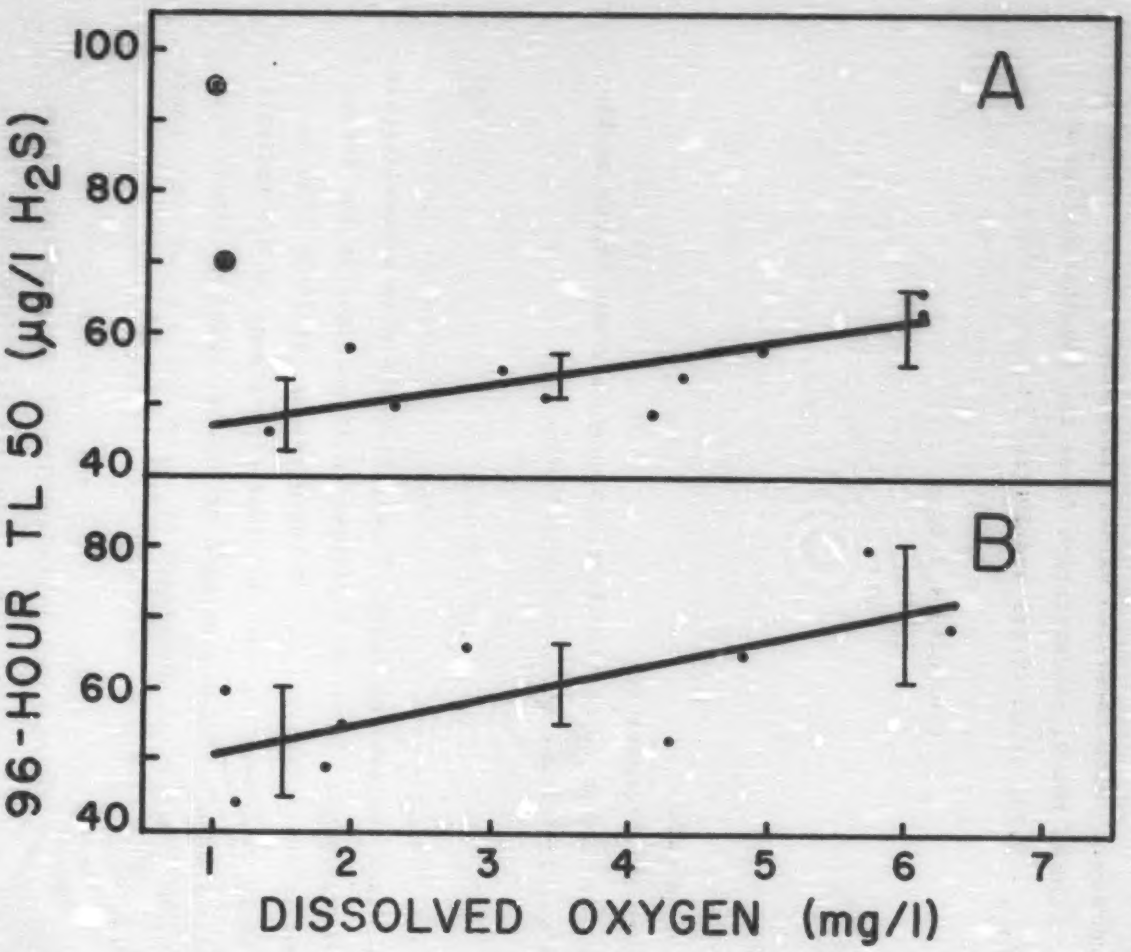

Figure 5. The effect of oxygen on the 96-hr LC50 (TL50) of H_2S to goldfish: (A) with oxygen acclimation prior to the bioassay and (B) without prior oxygen acclimation. The circled points are not included in the regression calculation, and 95% confidence limits are spaced at uniform intervals.

However, the actual difference in elevation of the regression lines is small and since there is a great variation in individual points, acclimation to oxygen does not affect H_2S toxicity appreciably, except at very low oxygen concentrations.

Tests of Bioassay Variability

In the series of tests of bioassay variability the means and standard deviations for temperature, pH meter readings, oxygen, and total alkalinity for 21 bioassays at each temperature were:

	Diluter 1	Diluter 2	Diluter 3
Temperature (C)	14.1 ± 0.12	20.1 ± 0.12	25.9 ± 0.10
pH meter reading	7.65 ± 0.02	7.64 ± 0.04	7.65 ± 0.05
Oxygen (mg/liter)	8.04 ± 0.43	7.96 ± 0.40	7.56 ± 0.31
Total alkalinity (mg/liter)	216.0 ±17	210.0 ± 9	158.0 ± 9

A summary of the LC50 values for these juvenile goldfish is presented in Table 41. Mean 4-day LC50's at 14, 20, and 26 C were 0.145, 0.083, and 0.063 mg/liter, respectively, and mean 11-day LC50's were 0.084, 0.071, and 0.060 mg/liter H_2S, respectively.

The 3 x 3 factorial design of the experiment in variability permitted analysis for differences due to acclimation time, temperature, and fish stocks. Since the pH and fish size may affect the toxicity of H_2S and could not be held precisely constant in different bioassays, an analysis of covariance (Snedecor and Cochran[13]) with pH and weight of the fish as covariates was used for both the 4-day and 11-day LC50 values (Table 42).

There was a significant difference in 4-day and 11-day LC50 values in tests conducted at different temperatures: $F_{2,43} = 167.27$ ($p < .01$) for the former and $F_{2,44} = 39.23$ ($p < .01$) for the latter (Figure 4). No significant difference in the 4-day or 11-day LC50 values was noted in

Table 41. EFFECT OF TEMPERATURE, ACCLIMATION TIME, AND GOLDFISH STOCK ON THE 96-HR AND 11-DAY LC50

(mg/liter H_2S)

Temperature, C	Acclimation time, weeks	Fish stock 1	2	3	4	5	6	7
14	1	0.120 (0.162)	0.081 (0.125)	0.080 (0.156)	0.082 (0.142)	0.094 (0.124)	0.080 (0.118)	0.073 (0.129)
	3	0.085 (0.126)	0.078 (0.150)	0.079 (0.132)	0.079 (0.163)	0.103 (0.195)	0.087 (0.152)	0.075 (0.180)
	5	0.082 (0.163)	0.068 (0.140)	0.066 (0.132)	0.080 (0.134)	0.096 (0.155)	0.099 (0.150)	0.076 (0.112)
20	1	0.076 (0.081)	0.065 (0.076)	0.077 (0.080)	0.078 (0.083)	0.075 (0.082)	0.057 (0.086)	0.066 (0.068)
	3	0.079 (0.115)	0.074 (0.098)	0.073 (0.089)	0.084 (0.092)	0.067 (0.081)	0.055 (0.068)	0.052 (0.071)
	5	0.081 (0.087)	0.076 (0.082)	0.072 (0.077)	0.079 (0.088)	0.084 (0.094)	0.061 (0.076)	0.069 (0.076)
26	1	0.054 (0.054)	0.052 (0.057)	0.056 (0.058)	0.055 (0.064)	0.062 (0.076)	0.064 (0.071)	0.050 (0.050)
	3	0.065 (0.071)	0.052 (0.052)	0.051 (0.053)	0.061 (0.063)	0.060 (0.064)	0.084 (0.084)	0.046 (0.055)
	5	0.063 (0.066)	0.063 (0.064)	0.065 (0.068)	0.056 (0.057)	0.066 (0.066)	0.072 (0.072)	0.055 (0.059)

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[a] 96-hr LC50 in parentheses.

Table 42. ACUTE MORTALITY OF GOLDFISH AT THREE TEMPERATURES EXPRESSED AS 96-HR AND 11-DAY LC50[a] (mg/liter H_2S)

	Temperature, C		
	14	21	26
96-hr LC50			
Mean	0.145	0.083	0.063
Range	0.112-0.195	0.068-0.115	0.050-0.084
Coefficient of variability	14%	13%	14%
11-day LC50[b]			
Mean	0.084	0.071	0.060
Range	0.066-0.120	0.052-0.084	0.046-0.084
Coefficient of variability	15%	13%	14%

[a] Results from 21 bioassays at each temperature.

[b] The 11-day LC50 is equivalent to the lethal threshold concentration at 20 and 26 C and approaches the LTC at 14 C.

bioassays conducted after 1, 3, or 5 weeks of temperature acclimation with the 4-day LC50's, $F_{2,43} = 2.56$ (n.s. at .05) and with the 11-day LC50's, $F_{2,44} = .76$ (n.s. at .05). Brett[14] showed that acclimation of goldfish to increased temperature as measured by their thermal tolerance proceeded at different rates depending on the range in which the increase occurred. An increase from 4 to 12 C required 20 days for acclimation to the higher temperature whereas the same increase from 20 to 28 C required 3 days. An increase from 12 to 20 C required 7 days of acclimation. This rise is similar to that in one group of fish of the present study where temperature was increased from approximately 12 to 20 C. Since these goldfish should require 7 days to acclimate in accordance with Brett's data, it might be expected that those fish raised from 12 to 26 C would take longer with a resulting increase in LC50's after the third or fifth week of acclimation. Fish stocks 1, 2, and 3 tested at 26 C became more tolerant to H_2S after 3 or 5 weeks of acclimation, but stocks 4-7 did not show this trend (Table 41). Stocks 4-7, obtained April-September, experienced seasonal mean temperatures ranging from approximately 12 to 26 C in the supplier's holding pond, and stocks 2 and 3, obtained in January and February, were exposed to pond temperatures of about 4-7 C. This exposure to cold temperatures may have resulted in a longer acclimation time in the experiments. Stock 1, obtained in November, was exposed to pond temperatures of about 13 C. This stock would be expected to respond as stocks 4-7 with no increased acclimation time, but increased time was required.

The analysis of covariance indicated that there was no significant difference between the 4-day LC50 values for the seven stocks of fish ($F_{6,43} = 1.57$, n.s. at .05), but there was a significant difference between the 11-day LC50 values ($F_{6,44} = 4.98$, $p < .01$). Stocks that are different in their overall resistance to a toxicant after 11 days may have similar rates of mortality during the earlier period of bioassay. Handling and sudden exposure to the toxicant may have increased the initial rates of mortality sufficiently in these bioassays to mask differences between stocks, and thus longer exposure was requried to show real differences.

To test the effect of temperature on the variability of the results, the coefficient of variability was computed from the 4-day and 11-day LC50's for each temperature. At 14, 20, and 26 C the coefficient of variability of the 4-day LC50 was 14, 13, and 14%, respectively, and of the 11-day LC50 was 15, 13, and 14%, respectively, indicating no essential difference in the variability due to temperature (Table 42).

Summary of Acute Tests

The increased resistance of the goldfish to H_2S at lower temperatures is probably of adaptive advantage. In the summer naturally occurring H_2S is generally restricted to the hypolimnion of lakes, so that fish may escape to the upper water strata. However, in shallow ice-covered lakes H_2S may be found at most all depths and may contribute to winter fish kills (Scidmore[15]). Under these conditions the goldfish could survive higher H_2S concentrations than in the summer when escape would be possible. If H_2S persists for a long period during the winter, deleterious sublethal effects may occur, but present data does not permit any predictions of effects of temperature.

Since H_2S usually occurs only at relatively low oxygen concentrations, its toxicity at these levels may have important environmental significance. However, the rate of increased toxicity with lowered oxygen is not very great, with the 96-hr LC50 reduced only about 26% between oxygen concentrations of 6 and 1 mg/liter. Furthermore, the difference in H_2S concentrations between bioassay chambers where all or none of the fish died was small. A mean H_2S concentration of 0.087 mg/liter caused 100% mortality and a mean of 0.036 mg/liter caused 0% mortality. Since complete mortality or survival occurs within a narrow range of H_2S and since the rate of decreased resistance with lowered oxygen is small, the oxygen concentration will not have an important effect on H_2S toxicity in the environment. When H_2S concentrations are greater than about 0.036 mg/liter at 17.5 C some goldfish mortality will probably occur regardless of the oxygen concentration.

The analysis of factors that could affect bioassay variability shows that prior acclimation of goldfish to the oxygen concentration of the bioassay had little effect on acute H_2S toxicity. One week of acclimation to temperatures of 14, 20, and 26 C appeared adequate. However, goldfish that experience different thermal histories than the stocks tested may require a longer time to acclimate. Fish held in winter conditions and tested at high temperatures and fish held in summer conditions and tested at low concentrations should be acclimated for a longer period of time.

The toxicity of H_2S to different stocks of fish was not the same although early mortality rates were similar. It is believed that this difference may relate to the season of year when fish were tested rather than genetic differences.

Although the range in 4-day and 11-day LC50's was greater at colder temperatures, the coefficient of variability was essentially the same at 12, 20, and 26 C. Therefore, variability of bioassay results is not affected by temperature in that range.

CHRONIC TESTS

Experimental Design

Three chronic bioassays with goldfish were conducted, two at the same time and in the same apparatus but with different stocks of fish and a third separate bioassay. The bioassays were designated Chronic I-O, Chronic I-LM, and Chronic II. The three bioassays were conducted in the modified proportional diluter. In Chronic II the control and four treatment chambers were galvanized metal stock watering tanks painted with an asphalt-aluminum roof coating. The tanks were 122 x 45.7 x 30.5

cm deep and fitted with a drain to keep the water level at 21.6 cm containing 123 liters. The same tanks were used in Chronic I but were divided by a longitudinal glass partition so that two stocks of fish were kept separately in one tank. A flow-splitter divided the incoming water equally between each side.

The goldfish tested in Chronic I-O were obtained from Ozark Fisheries, Inc. The fish were 18 months old at the start of the bioassay, and their mean weight was 2.96 g (range 1.56-5.30). Fish in Chronic I-LM were obtained from the federal hatchery at Lake Mills, Wisconsin. They were 6 months old and weighed 3.56 g (range 1.80-6.36) at the start. The Lake Mills and Ozark fish were assigned in a stratified random manner to the appropriate section of each experimental chamber, 20 fish per chamber. After approximately 4 months fish were randomly thinned, Ozarks to 13 and Lake Mills to 14 fish per tank. The fish obtained from thinning were tested for resistance to acute malathion and acute H_2S toxicity. When fish began active spawning, artificial tropical fish spawning grass made of plastic strands entwined in a stainless steel wire was provided to each tank. All fish were fed Oregon moist trout pellets *ad libitum* three times each day.

The Chronic I bioassay lasted 294 days and a cyclic temperature regime was used. The initial temperature of approximately 14 C was held for 1 month, then raised to 19 C for 3 months, raised to about 22 C for 2 months, lowered to about 13 C for 1-1/2 months, and finally raised to 19 C for 2 months. The H_2S and dissolved oxygen concentrations varied slightly between the Lake Mills and Ozark sections of the tank, probably due to differences in the fish's metabolism or flow characteristics (Table 43). The extreme range of O_2 resulted from conditions occurring only occasionally.

Chronic II was conducted for 430 days starting with eggs. Four male and four female 2-year-old Ozark goldfish were artificially spawned. Five

Table 43. TEST CONDITIONS IN GOLDFISH CHRONIC I

Lake Mills Goldfish					
H_2S (mg/1) - mean	Control	0.002	0.005	0.010	0.025
std. dev.	-	0.0009	0.0022	0.0050	0.0105
pH - mean	7.61	7.51	7.52	7.56	7.57
range	7.10-8.01	7.20-7.96	7.01-7.92	7.10-8.03	7.12-8.01
Temperature (C)-mean	18.5	18.4	18.9	18.5	18.7
range	12.8-24.0	12.8-23.7	13.2-24.1	12.8-23.8	13.0-23.9
Dissolved O_2 (mg/1)					
mean	6.33	5.31	5.24	5.23	5.54
range	2.60-10.55	2.20-9.90	2.00-9.45	2.30-9.90	2.75-10.95
Total alkalinity					
(mg/1 $CaCO_3$) -mean	184	184	184	184	184
range	162-230	162-230	162-230	162-230	162-230
Ozark Goldfish					
H_2S (mg/1) - mean	Control	0.002	0.005	0.010	0.028
std. dev.	-	0.0010	0.0023	0.0047	0.0125
pH - mean	7.61	7.51	7.52	7.56	7.57
range	7.10-8.01	7.02-7.96	7.01-7.92	7.10-8.03	7.12-8.01
Temperature (C) -mean	18.5	18.4	18.9	18.5	18.7
range	12.8-24.0	12.8-23.7	13.2-24.1	12.8-23.8	13.0-23.9
Dissolved O_2 (mg/1)					
mean	6.65	4.96	4.84	5.31	5.39
range	2.65-10.40	2.20-9.85	1.35-9.35	2.35-9.75	2.75-10.80
Total alkalinity					
(mg/1 $CaCO_3$) -mean	184	184	184	184	184
range	162-230	162-230	162-230	162-230	162-230

hours after fertilization 20 fertile eggs from each spawning were placed in each experimental chamber for a total of 80 eggs. The egg chambers are described in the section on acute bioassays of eggs. On the third day after hatching the fry were thinned to 50 per tank. Since complete mortality of eggs occurred in the highest H_2S concentration, 25 fry from each of the two lowest H_2S concentrations were placed in this chamber. Young fry were fed egg yolk, brine shrimp, and ground Glencoe trout pellets. Fry chambers at this time were glass tanks 30 x 15 x 30 cm deep, containing 10 liters. On the 33rd day after hatching the fish were again thinned and transferred, 30 per chamber, to a 38-liter glass aquarium. After 125 days all fish were again transferred to the stock watering tanks. From this time on the fish were fed Oregon moist trout pellets of various sizes, ad libitum. After 304 days a final thinning left 15 fish per chamber.

A constant temperature of approximately 22 C occurred for all but 15 days late in the bioassay when the temperature was lowered to approximately 13 C, then raised again in an attempt to induce spawning. The chemical conditions in Chronic II are summarized in Table 44.

Eggs and Fry

Data on eggs and fry were obtained in Chronic II. The effect of H_2S on eggs was reported in the acute bioassay section (Table 37). No increased mortality over controls occurred at 0.0052 and 0.0097 mg/liter H_2S, but at 0.0169 mg/liter mortality was 13% greater and at 0.0291 mg/liter, 75% greater than controls. These latter two H_2S concentrations also caused malformed fry at hatching, 14% at 0.0169 mg/liter and 62% at 0.0291 mg/liter (Table 37).

Since nearly all eggs had died at the highest H_2S concentration, the fry tested at this concentration were hatched from eggs in the two lowest concentrations. No mortality of fry occurred at any H_2S concentration tested (Table 44).

Table 44. TEST CONDITIONS IN GOLDFISH CHRONIC II

H_2S (mg/l) - mean	Control	0.007	0.009	0.015	0.024
std. dev.	-	0.0029	0.0072	0.0056	0.0186
pH - mean	7.62	7.59	7.57	7.63	7.63
range	7.38-8.02	7.40-8.02	7.33-8.18	7.39-8.10	7.30-7.99
Temperature (C)-mean	21.4	21.4	21.5	21.6	21.4
range	12.4-25.7	12.4-26.0	12.3-25.7	12.3-26.0	12.4-25.7
Dissolved O_2 (mg/l)					
mean	5.93	5.95	5.47	6.13	6.62
range	2.05-9.00	2.00-9.25	2.85-9.65	2.50-9.05	2.00-8.25
Total alkalinity					
(mg/l $CaCO_3$)-mean	194	194	194	194	194
range	178-225	178-225	178-225	178-225	178-225

Growth Rate

The goldfish were weighed at monthly intervals in the three chronic bioassays. At termination of Chronic I-LM, only the fish in the highest concentration weighed less than the controls and intermediate concentrations stimulated growth (Table 45). The no-effect level of H_2S on growth was between 0.010 and 0.025 mg/liter. In Chronic I-O intermediate levels also stimulated growth with only the highest H_2S concentration weighing less than the control at termination (Table 45). The level of no effect with the Ozark fish was between 0.010 and 0.028 mg/liter H_2S.

In Chronic II the mean weights decreased at all increasing H_2S concentrations before thinning at 308 days and at termination, 430 days (Table 45). The analysis of variance indicated a significant difference at both times, $F_{4,138} = 8.34$, $p < .01$, at the earlier time and $F_{4,68} = 5.28$, $p < .01$, at the final weighing. The test of least significant difference showed that the level of effect occurred at one lower H_2S concentration by the end of the test than at 308 days. The lowest concentration significantly different than the control was 0.0616 mg/liter H_2S at 308 days, but after 430 days it was 0.009 mg/liter. The no-effect level was between 0.007 and 0.009 mg/liter H_2S (Table 46).

Reproduction

Spawning occurred only in the Chronic I-O bioassay. The largest number of spawnings and spawnings per female occurred at 0.005 mg/liter H_2S (Table 47). No spawning occurred at 0.002 mg/liter, but there was only one immature female in that tank. One spawning per female occurred in the control, but there were only two females in this tank compared to four in the three highest treatments. Fish in the two highest treatments spawned at a reduced rate compared to spawning at 0.005 mg/liter even though the tanks contained the same number of females. It appears, therefore, that the safe level for optimum frequency of spawning is between 0.005 and 0.010 mg/liter H_2S (Table 47).

Table 45. WEIGHTS OF GOLDFISH IN THREE CHRONIC BIOASSAYS (mean weight in grams)

Time of weighing	Days from start	H_2S concentration, mg/liter				
Chronic I-LM		0	0.002	0.005	0.010	0.025
Start	0	3.36	3.72	3.47	3.51	3.74
Before thinning	120	13.41	15.74	14.77	14.36	11.39
End	294	38.09	48.34	46.60	46.70	31.40
Chronic I-O		0	0.002	0.005	0.010	0.028
Start	0	2.75	2.93	2.91	3.10	3.09
Before thinning	120	11.99	14.98	13.84	14.10	10.91
End	294	32.28	36.68	33.14	38.97	26.77
Chronic II		0	0.007	0.009	0.015	0.024
Before thinning	30	0.12	0.10	0.13	0.10	0.09
Before thinning	308	42.03	40.64	34.76	30.94	20.32
End	430	78.68	67.05	56.81	54.76	34.85

Table 46. H_2S CONCENTRATIONS NOT AFFECTING GOLDFISH ADVERSELY DETERMINED FROM THREE CHRONIC BIOASSAYS
(mg/liter)

Bioassay	Basis of determination	Highest level of no effect	Lowest level of measured effect
Chronic I-IM	Final weight	0.010	0.025
Chronic I-O	Final weight	–[a]	–[a]
	Spawnings per female	0.005	0.010
Chronic II	Egg survival	0.010	0.017
	Weight after 308 days	0.009	0.016
	Final weight	0.007	0.009
Estimate from all effects		0.005	0.009

[a] No significant difference between highest level of no effect and lowest level of measured effect.

Table 47. REPRODUCTION OF GOLDFISH IN CHRONIC I-O

	H_2S concentration, mg/liter				
	0	0.002	0.005	0.010	0.025
Number of males	9	12	9	8	7
Number of females	2	1	4	4	4
Number of spawnings	2	0	12	2	3

Since the goldfish scattered their eggs among the artificial grass strands and since non-spawning fish ate many eggs, it was not possible to make an accurate estimate of fecundity. Attempts to determine survival of spawned eggs and fry were unsuccessful due to technique and apparatus malfunction.

Other Chronic Effects

The fish from Chronic I-0 that were removed after thinning at 120 days were subjected to two acute treatments. Five fish from each H_2S concentration were subjected to 1.0 mg/liter malathion for 96 hr to determine if chronic exposure to H_2S affected their tolerance to another toxicant. The level of malathion was ineffective since no mortality occurred among any goldfish.

After this treatment the same fish remained in pure well water for 3 days and then were subjected to 0.322 mg/liter H_2S until all fish died by 135 hr of exposure. The time of death of each fish was recorded. It appeared that those fish exposed to the highest H_2S concentration were the least resistant to an acutely toxic H_2S concentration (Table 48), however an analysis of variance indicated no significant difference ($F_{4,20} = 1.27$).

SUMMARY

In acute tests egg survival decreased at 0.017 mg/liter H_2S and above. The LC50 at hatching was 0.022 mg/liter. LC50 at 96 hr for fry was 0.025 mg/liter. Ninety-six-hr LC50 for juveniles varied from 0.556 mg/liter at 6.7 C to 0.044 mg/liter at 25 C. At different oxygen levels 96-hr LC50 varied from 0.071 at 6.0 mg/liter O_2 to 0.053 mg/liter H_2S at 1.5 mg/liter O_2 at 17.5 C. In chronic tests the level of H_2S not adverse to growth is between 0.007 and 0.009 mg/liter H_2S. The number of spawnings per female was not affected between 0.005 and 0.010 mg/liter H_2S.

Table 48. TIME TO DEATH OF GOLDFISH FROM VARIOUS CHRONIC H_2S CONCENTRATIONS EXPOSED TO AN ACUTELY LETHAL H_2S CONCENTRATION OF 0.322 MG/LITER
(hours)

Order of mortality	Chronic H_2S concentration, mg/liter				
	0	0.002	0.005	0.010	0.025
1	76	100	79	88	84
2	83	106	102	89	90
3	103	127	106	114	94
4	122	127	116	115	101
5	124	127	135	126	102
	508	587	538	532	471

SECTION VII

BLUEGILL

(*Lepomis macrochirus* Rafinesque)

Toxicity of H_2S to bluegills was determined by (1) a series of acute tests on eggs, fry, juveniles and adults, (2) an acute test on juveniles acclimated to H_2S, and (3) a series of chronic and partial chronic tests.

ACUTE BIOASSAY

Experimental Design

Acute toxicity tests were run on bluegill eggs, sac fry, juveniles, and adults. A total of two tests on green eggs, one on newly hatched sac fry, one on 35-day-old fry, eight on young-of-the-year (yy), and seven on adult bluegills were conducted (Table 49). Green eggs were stripped in the laboratory from adults taken in the field. Eggs were fertilized and tests were started on the same day. Sac fry were incubated at 22 C from eggs stripped and fertilized in the laboratory and were placed in test chambers within 24 hr after hatching. Thirty-five-day-old fry were reared in the laboratory from natural spawning in laboratory tanks. Fry were fed mashed hard boiled egg yolk, brine shrimp, ground beef liver, Glencoe dry granules, and ground minnows. Juvenile fish were seined in the field, brought to the laboratory, and treated for 3 consecutive days with 20 mg/liter neomycin, then with 2.65 mg/liter methylene blue for the following 3 days. All fish were held at 21 C and fed Oregon moist pellets, Glencoe dry fry granules, brine shrimp, and ground pork liver prior to tests. Adults were secured in the field,

Table 49. SOURCE OF BLUEGILLS AND STAGE OF FISH USED FOR ACUTE TESTS WITH H_2S

Test	Stage	Date collected	Water temperature at collection, C	Method of capture	Source
BE-1	Green egg	1/7/71	24	Adults by hook and line	Carnelian Lake
BE-3	Green egg	13/7/71	23	Adults by hook and line	Pleasant Lake
BF-2	Sac fry	13/7/71	23	Adults by hook and line	Pleasant Lake
BF-4	35-day fry				Laboratory reared
Lmyy-1	yy	26/9/68	20	Seine	Medicine Lake
-2	"	"	"	"	" "
-3	"	"	"	"	" "
-4	"	7/10/69	17	"	" "
-5	"	"	"	"	" "
-7	"	"	"	"	" "
-8	"	3/10/69	18	"	" "
-9	"				Laboratory reared
Lma-1	Adults	8/11/68	6	Trap net	Crystal Lake
-3	"	16/10/68	17	" "	" "
Lma-4	"	"	"	" "	" "
-5	"	22/1/69	4	Hook and line	" "
-6	"	"	"	" " "	" "
-7	"	10/2/73	"	" " "	" "
-10	"	30/9/70	18	Trap net	" "

brought to the laboratory, and treated prophylactically as described above for fingerlings, held at 13 C, fed Oregon moist pellets, Glencoe dry pellets, live minnows, fresh and frozen minnows, and ground pork liver. Adult fish were held 7 days at test temperature prior to test. On the basis of preliminary acute tests, H_2S concentrations for acute tests were set to bracket the probable LC50 value. Green egg tests had two controls and ten H_2S concentrations. One adult test, designated as Lma-7 (Table 49), also had two controls and ten H_2S concentrations. All other tests were conducted with one control and five H_2S concentrations. The light regime was 12 hr of light and 12 hr of darkness through the period of the test.

All acute tests were of the flow-through type with flushing water and toxicant provided for one control and five treatment chambers from apparatus described by Colby and Smith[1] and modified by Adelman and Smith.[2] This system used deoxygenated water and H_2S gas to introduce the toxicant to the test chambers. Acrylic test chambers for eggs and fry were similar to those described by Colby and Smith.[1] Young-of-the-year fish were tested in 36-liter slate-bottomed glass-walled aquaria, 6-liter round-bottomed acrylic hatchery jars (15 x 44 cm), or in 25-liter glass aquaria constructed with silicone glue. Adult fish were tested in the 36-liter aquaria described above. Tests designated as BE-1, BE-3, and Lma-7 had two controls and ten H_2S concentrations. During the acute test period all test chambers were subjected to 12 hr of light and 12 hr of darkness. Fish were not fed during the first 96 hours of test but were fed thereafter when the test duration was longer.

Acute Toxicity

Green Eggs—The two green egg tests (BE-1 and -3) were run at 21.9 C and 6.1 and 5.9 mg/liter O_2, respectively (Table 50). The eggs in the test run at 6.1 mg/liter O_2 hatched in 66 hr with an LC50 of 0.0162 mg/liter H_2S. The second test run at 5.9 mg/liter O_2 hatched in 77 hr with an LC50 of 0.0125 mg/liter H_2S. A mean LC50 (66-72 hr) for the two tests was 0.0144 mg/liter H_2S.

Sac Fry—Sac fry were tested at 21.7 C and 5.8 mg/liter O_2 4 days after egg fertilization and within 24 hr after hatch (BF-2). The 96-hr LC50 was greater than 0.0292 mg/liter H_2S (Table 30). After 9 days the LC50 was 0.0169 mg/liter H_2S. Advanced feeding fry were tested at 21.8 C and 6.0 mg/liter O_2 39 days after egg fertilization (BF-4). The 96-hr LC50 was 0.0086 mg/liter and after 8 days was 0.0084 mg/liter H_2S (Table 30).

Juveniles—Seven tests were run on juvenile bluegills ranging in size from 3.2 to 5.3 cm (Table 30). Temperatures at which the tests (Lmyy 4-9) were conducted varied from 20.1 to 19.9 C, with O_2 ranging from 5.7 to 6.6 mg/liter. The 96-hr LC50 values varied from 0.0290 to 0.0325 mg/liter H_2S with a mean value for these tests of 0.0316 mg/liter. One test (Lmyy-7) run for 8 days to threshold LTC was 0.0325 mg/liter H_2S and a second run to 10 days (Lmyy-9) was 0.0310 mg/liter. Fish in tests Lmyy-1 and -2 with a mean 96-hr LC50 of 0.0222 mg/liter H_2S may have a greater sensitivity than would be normally expected since fish from the same stock held in fresh water developed an infection of "ich" several days after the completion of the test.

Adult Fish—Seven acute tests were run on adult bluegills held in fresh laboratory water for 6 to 174 days prior to bioassay. Fish in the various tests had mean lengths varying from 11.6 to 13.0 cm. Temperatures in various tests were 19.6 to 20.3 C and O_2 levels were 4.6 mg/liter in one test ([illegible]-10) and in others ranged from 5.8 to 6.4 mg/liter. The 96-hr LC50 values ranged from 0.0196 to 0.0373 mg/liter H_2S with a mean for all tests of 0.0297 mg/liter (Table 30).

Effect of Acclimation to H_2S on Acute Response—It will be noted from Table 30 that LC50 values did not decline significantly after 48 hr exposure in most tests. To determine to what extent acclimation to H_2S

Table 50. ACUTE TEST CONDITIONS AND LC50 VALUES FOR BLUEGILLS TESTED IN H_2S

Test	Days from collection to start of test	Mean fish length, cm	Mean test conditions[a] Temp., C	Mean test conditions[a] O_2, mg/l	LC50 values, mg/l H_2S 48 hr	LC50 values, mg/l H_2S 72 hr	LC50 values, mg/l H_2S 96 hr	LC50 values, mg/l H_2S LTC(days)
BE-1	0	-	21.9	6.1	-	(66 hr) 0.0162	-	-
BE-3	0	-	21.9	5.9	-	(77 hr) 0.0125	-	-
BF-2	4[b]	0.3	21.7	5.8	-	-	>0.0292	0.0169(9)
BF-4	39[c]	0.8	21.8	6.0	-	-	0.0086	0.0084(8)
Lmyy-1[d]	60	3.7	19.8	6.3	-	-	0.0173	-
Lmyy-2[d]	67	3.8	19.8	6.5	-	-	0.0270	-
							$\bar{x}$ = 0.0222	
Lmyy-4	28	3.2	20.1	6.6	0.0345	0.0345	0.0325	-
Lmyy-5	42	3.2	20.1	6.3	0.0345	0.0320	0.0320	-
Lmyy-7	99	3.2	20.0	5.9	0.0340	0.0325	0.0325	0.0325(8)
Lmyy-8	164	4.9	19.9	5.9	0.0290	0.0290	0.0290	-
Lmyy-9	262[e]	5.3	20.0	5.7	-	-	0.0320	0.0310(10)
							$\bar{x}$ = 0.0316	
Lmm-1	31	12.0	19.8	6.3	0.0237	0.0195	0.0198	-
Lmm-3	45	12.0	19.8	6.4	-	-	0.0375	-
Lmm-4	89	12.0	19.6	6.0	0.0317	0.0317	0.0280	-
Lmm-5	33	12.0	20.0	6.1	0.0280	0.0280	0.0280	-
Lmm-6	103	12.0	20.3	5.8	0.0270	0.0270	0.0270	-

Table 50 (continued). ACUTE TEST CONDITIONS AND LC50 VALUES FOR BLUEGILLS TESTED IN H_2S

Test	Days from collection to start of test	Mean fish length, cm	Mean test conditions[a] Temp., C	O_2, mg/l	LC50 values, mg/liter H_2S 48 hr	72 hr	96 hr	LTC(days)
Lms-7	174	13.0	20.2	5.8	0.0328	0.0328	0.0328	-
Lms-10	6	11.6	20.0	4.6	0.0353	0.0353	0.0351	-
							$\bar{x}$ = 0.0297	

[a] pH range 7.6–8.0.

[b] Days since spawned artificially in the laboratory from parents collected in the field.

[c] Days since spawned naturally in the laboratory from parents which were controls in chronic tests "Bluegill-small".

[d] Fish infected with *Ichthyophthirius multifiliis* and LC50 values not included with other yy tests.

might be a factor in these results, 10 test chambers were set up with serial concentrations of H_2S varying from 0.0144 to 0.0308 mg/liter (Table 51). On each succeeding day of the 11-day test, except days 6 and 7, the H_2S concentration was raised in each chamber. At the lowest starting concentration (0.0144 mg/liter), 100% of the fish survived 8 days or until the concentration had been raised to 0.0322 mg/liter. On the succeeding day with the concentration raised to 0.0502 mg/liter, 50% of the fish died and 100% died on the 11th day at a concentration of 0.0873 mg/liter. At the highest initial concentration of 0.0308 mg/liter, 25% of the fish survived at the end of the first day and with the increase in H_2S concentration on the second day to 0.0349 mg/liter, 100% mortality occurred. At intermediate starting concentrations the ultimate concentration at which 50% and 100% of the fish died increased with the decrease in starting concentration (Table 52). These data indicate that acclimation to H_2S occurred during the progress of the experiment provided that initial levels were not acutely toxic within 24 to 48 hr. This experiment also suggests the reason why the 48-hr and longer LC50 values in tests reported above did not vary significantly (Table 32).

The acute tests of different life history stages of bluegill indicate that feeding swim-up fry with a 96-hr LC50 of 0.0086 mg/liter H_2S are the most sensitive and that the most resistant is the juvenile stage. The apparent difference between juvenile and adult resistance was not shown to be significant.

CHRONIC BIOASSAY

Experimental Design

Eight chronic tests were conducted and varied in duration from 93 to 826 days. Fish were started in the various tests as green eggs, young-of-the-year, or adults (Table 33). H_2S concentrations ranged from means of 0.0007 to 0.0103 mg/liter and temperatures from 24.4 to 14.7 C, O_2 from

6.2 to 9.0 mg/liter, and pH from 7.58 to 8.08 in the various tests (Table 34). In one test a seasonal temperature variation was developed. The criteria for judging effect levels were growth rate, long-term mortality, food consumption, food conversion efficiency, and reproductive success. At the very low levels variation in concentration was considerable in some tests but the mean condition was sustained the greater part of the time. One additional chronic test was made to determine the effect of sublethal exposure to H_2S on swimming endurance and resistance to copper.

All chronic tests were of flow-through type with flushing water provided by diluters modified from that described by Mount and Brungs[3] and Brungs and Mount.[4] Rate of flow was 0.5 liter per minute. When fish were less than 2 cm long they were fed yeast, finely mashed hard boiled egg yolk, and brine shrimp. After fish exceeded 2 cm in total length, yeast and egg yolk were discontinued and the diet changed to ground beef liver, ground fresh minnows, Glencoe dry fry granules, and brine shrimp. The test chambers varied in size depending on the size of the fish. BG-1, -2, and -3, and BG-small were tested in 303-liter insulated fiber glass tanks (208 x 35 x 52 cm) with water depth carried at 44 cm. Bluegill fry (BG-sp 1 & 2) were started in a 20-liter glass-silicone glue aquarium and after 145 days of exposure, fish were transferred to the 303-liter tanks described above. Bluegill in tests BG-sp 3 & 4 and BG-sp 5 & 6 were tested in 20-liter glass aquaria. Light regime was regulated in accordance with seasonal changes in the St. Paul, Minnesota area.

Survival in Chronic Exposure

Three chronic tests ranging in duration from 93 to 316 days were started with green eggs hatched in H_2S concentrations which varied from 0.0010 to 0.0092 mg/liter in the various tests (Table 34). Percentage survival in the controls at temperatures from 22 to 24 C was 39% and 33% after 316 and 130 days, respectively, in tests BG-sp 1 & 2 and 3 & 4 (Table 35). After initial egg and fry mortality, survival decreased

TABLE [illegible]. MEAN DAILY H_2S EXPOSURE AND PERCENTAGE SURVIVAL OF FINGERLING BLUEGILLS ON SUCCESSIVE DAYS IN 10 TREATMENT CHAMBERS

(H_2S concentration expressed as mg/liter)

	Days of exposure										
	1	2	3	4	5	6	7	8	9	10	11
H_2S	0.0144	[illegible]	[illegible]	[illegible]	0.0325	0.0325	0.0325	0.0332	[illegible]	0.0671	0.0873
% survival	100	100	100	100	100	100	100	100	50	50	0
H_2S	0.0171	[illegible]	0.0355	0.0381	0.0460	0.0460	0.0460	0.0438	0.0612	0.0877	-
% survival	100	100	100	100	100	75	75	75	25	0	-
H_2S	0.0179	[illegible]	0.0325	0.0400	0.0455	0.0455	0.0455	0.0446	[illegible]	-	-
% survival	100	100	100	100	100	75	75	75	0	-	-
H_2S	[illegible]	[illegible]	[illegible]	0.0467	0.0361	0.0361	0.0361	0.0551	[illegible]	0.0658	-
% survival	100	100	100	100	100	100	100	100	50	0	-
H_2S	[illegible]	[illegible]	[illegible]	0.0480	0.0336	0.0336	0.0336	0.0435	[illegible]	0.0560	0.0774
% survival	100	100	100	100	100	100	100	100	100	50	0
H_2S	[illegible]	[illegible]	[illegible]	-	-	-	-	-	-	-	-
% survival	[illegible]	25	0	-	-	-	-	-	-	-	-
H_2S	[illegible]	[illegible]	[illegible]	[illegible]	0.0361	0.0361	0.0361	0.0558	-	-	-
% survival	[illegible]	[illegible]	[illegible]	25	25	25	25	0	-	-	-
H_2S	[illegible]	[illegible]	[illegible]	0.0446	0.0403	0.0403	0.0403	0.0541	[illegible]	-	-
% survival	100	100	100	100	100	100	100	100	0	-	-

Table 51 (continued). MEAN DAILY H_2S EXPOSURE AND PERCENTAGE SURVIVAL OF FINGERLING BLUEGILLS ON SUCCESSIVE DAYS IN 10 TREATMENT CHAMBERS

(H_2S concentration expressed as mg/liter)

	Days of exposure										
	1	2	3	4	5	6	7	8	9	10	11
H_2S	0.0284	0.0336	0.0389	0.0533	-	-	-	-	-	-	-
% survival	75	50	25	0	-	-	-	-	-	-	-
H_2S	0.0308	0.0349	-	-	-	-	-	-	-	-	-
% survival	25	0	-	-	-	-	-	-	-	-	-

Table 52. H_2S CONCENTRATIONS AT WHICH 100%, 50%, and 0% OF BLUEGILLS SURVIVED IN 2 TO 11 DAYS AFTER ACCLIMATION TO H_2S

(H_2S concentration expressed as mg/liter, days exposure in parentheses)

Initial concentration	% Survival (24 hr)	Highest concentration with 100% survival	Highest concentration with 50% survival	Highest concentration with 0% survival
0.0144	100	0.0332 (8)	0.0671 (10)	0.0873 (11)
0.0171	100	0.0460 (5)	0.0525 (8.5)[a]	0.0877 (10)
0.0179	100	0.0455 (5)	0.0623 (8.5)[a]	0.0800 (9)
0.0205	100	0.0551 (8)	0.0582 (9)	0.0568 (10)
0.0219	100	0.0560 (9)	0.0560 (10)	0.0774 (11)
0.0231	100	0.0231 (1)	0.0313 (1.5)[a]	0.0401 (3)
0.0247	50	<0.0247 (<1)	0.0247 (1)	0.0568 (8)
0.0276	100	0.0541 (8)	0.0720 (8.5)[a]	0.0898 (9)
0.0284	75	<0.0284 (<1)	0.0336 (2)	0.0533 (4)
0.0308	25	<0.0308 (<1)	0.0308 (<1)	0.0349 (2)

[a] Interpolated value.

Table 53. SOURCE OF BLUEGILLS AND STAGE OF FISH AT START OF CHRONIC TEST WITH H_2S

Test	Stage	Date collected	Water temperature at collection, C	Method of capture	Source
BG-sp 1 & 2	Green egg	27/5/71	-	Laboratory spawned	Laboratory
BG-sp 3 & 4	Green egg	10/6/71	-	Laboratory spawned	Laboratory
BG-sp 5 & 6	Green egg	22/6/71	-	Laboratory spawned	Laboratory
BG-small	yy	27/8/69	17	Seine	Medicine Lake
BG-1	Adult	30/1/69	4	Hook & line	Crystal Lake
BG-2	Adult	10/2/70	4	Hook & line	Crystal Lake
BG-4	Adult	10/5/72	15	Trap net	Marion Lake

Table [illegible]. [illegible]

[illegible] (316 days)					
x̄ H_2S concentration (mg/l)	-	0.0021	0.0042	0.0075	0.0082
H_2S std. dev. (mg/l)	-	0.0012	0.0029	0.0041	0.0042
x̄ pH	7.70	7.70	7.70	7.73	7.72
x̄ temperature (C)	[illegible]	22.4	[illegible]	[illegible]	[illegible]
x̄ dissolved O_2 (mg/l)	7.6	7.4	[illegible]	[illegible]	[illegible]
x̄ total alkalinity (mg/l)	[illegible]	195	195	[illegible]	[illegible]
[illegible] (180 days)					
x̄ H_2S concentration (mg/l)	-	0.0012	[illegible]	[illegible]	[illegible]
H_2S std. dev. (mg/l)	-	[illegible]	[illegible]	[illegible]	[illegible]
x̄ pH	[illegible]	[illegible]	7.73	[illegible]	[illegible]
x̄ temperature (C)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]					
x̄ H_2S concentration (mg/l)	-	[illegible]	[illegible]	[illegible]	[illegible]
H_2S std. dev. (mg/l)	-	[illegible]	[illegible]	[illegible]	[illegible]
x̄ pH	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
x̄ temperature (C)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
x̄ dissolved O_2 (mg/l)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]					
x̄ H_2S concentration (mg/l)	-	[illegible]	[illegible]	[illegible]	[illegible]
H_2S std. dev. (mg/l)	-	[illegible]	[illegible]	[illegible]	[illegible]
x̄ pH	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
x̄ temperature (C)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
x̄ dissolved O_2 (mg/l)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
x̄ total alkalinity (mg/l)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]					
x̄ H_2S concentration (mg/l)	-	[illegible]	[illegible]	[illegible]	[illegible]
H_2S std. dev. (mg/l)	-	[illegible]	[illegible]	[illegible]	[illegible]
x̄ pH	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
x̄ temperature (C)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 54 (continued). PHYSICAL AND CHEMICAL CONDITIONS OF CHRONIC BLUEGILL TESTS

	BG-1 Adult (288 days)				
$\bar{x}$ dissolved O_2 (mg/l)	6.3	6.3	6.3	6.3	6.3
$\bar{x}$ total alkalinity (mg/l)	232	232	232	232	232
	BG-2 Adult (200 days)				
$\bar{x}$ H_2S concentration (mg/l)	-	0.0010	0.0025	0.0062	0.0105
H_2S std. dev. (mg/l)	-	0.0007	0.0014	0.0046	0.0103
$\bar{x}$ pH	7.58	7.60	7.61	7.63	7.67
$\bar{x}$ temperature (C)[c]	14.7	14.9	15.2	14.9	15.2
$\bar{x}$ dissolved O_2 (mg/l)	7.9	7.9	7.9	7.9	7.9
	BG-4 Adult (97 days)				
$\bar{x}$ H_2S concentration (mg/l)	-	0.0007	0.0014	0.0027	0.0078
H_2S std. dev.	-	0.0007	0.0020	0.0048	0.0121
$\bar{x}$ pH	7.82	7.81	7.82	7.87	7.85
$\bar{x}$ temperature (C)[d]	23.9	23.8	23.6	23.6	23.5
$\bar{x}$ dissolved O_2 (mg/l)	6.4	6.3	6.2	6.4	6.5
$\bar{x}$ total alkalinity (mg/l)	181	181	181	181	181

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[a] Eggs composited from two spawnings of same parents.

[b] Temperatures varied seasonally from 7.4-25.5 C.

[c] Temperatures varied seasonally from 6.8-24.3 C.

[d] Temperatures varied seasonally from 20.0-25.8 C.

little with time. Survival decreased progressively with increased concentration of H_2S to 14% and 11%, respectively, in 0.0092 and 0.0087 mg/liter H_2S at the end of the test (Table 55). A third test started with eggs and run for 93 days was not conclusive since excessive mortality occurred in the controls. However, survival at 0.0073 mg/liter H_2S was approximately half that in the controls. The no-effect concentration for maximum long-term survival when fish are exposed to H_2S starting with the egg stage is less than 0.0012 mg/liter H_2S.

The chronic test designated as BG-small was run for 826 days starting with juveniles having a mean weight of 4.02-5.18 g in various treatments after 56 days of exposure. H_2S concentrations were 0.0015 to 0.0064 mg/liter. Little mortality occurred at any level before 362 days (Table 55). At the highest level (0.0064 mg/liter), survival dropped to 60% after 392 days and to 0 after 420 days. At 0.0061 mg/liter, survival was 100% at 717 days and 70% by the 826th day. It is apparent that the safe range for fish exposed to H_2S first as juveniles is approximately 0.0061 mg/liter and that lethal ranges are somewhat higher. The difference in the means of the two highest concentrations are not considered to be definitive since the standard deviation of both was high.

Three tests of bluegills started as adults (BG-1, -2, and -4) were conducted with H_2S concentrations varying from 0.0007 to 0.0103 mg/liter. After 200 days there was 88% survival at 0.0098 mg/liter in one test, or approximately the same as the control. In a second test, survival decreased after 56 days to 83% at 0.0062 mg/liter and fell to about half of the controls (56%) at 0.0105 mg/liter. The loss at the higher concentration occurred at the end of 28 days and did not increase through the remainder of the 200-day test period. The third test with a range of treatments from 0.0007 to 0.0078 mg/liter H_2S ran for 97 days with no mortality. The low survival in the tests started with eggs appeared to be attributable to mortality occurring during the very early stages since once fish reached a mean weight of 4-5 g, mortality was low at levels as high as 0.0061 mg/liter H_2S.

Growth Rate

Growth rate of fish started as eggs in BG-sp 1 & 2 and BG-sp 3 & 4 which ran for 316 and 113 days, respectively, decreased at the higher levels but some apparent stimulation occurred at 0.0012 and 0.0018 mg/liter H_2S (Table 56). After 316 days of exposure to 0.0092 mg/liter, mean weight was 40.8% of control and after 113 days of exposure in a second test at 0.0087 mg/liter was 85% of control.

When fish were started as juveniles, growth was retarded at all H_2S levels tested above 0.0015 mg/liter. At 0.0061 mg/liter, weight was 63% of the control after 826 days but the reduced mean size at the end of the test was due primarily to mortality among the larger fish.

In the three tests with adult bluegills (BG-1, -2, and -4), significant decrease in growth did not occur at concentrations less than 0.0071 mg/liter H_2S after 288 days of exposure. The mean weight of fish held for 288 days at 0.0098 mg/liter was 61% of control. A test (BG-2), running a shorter period of time (200 days), showed marked decline only at 0.0105 mg/liter where mean weight was 73% of control. It appears that growth inhibition will occur after long exposure to all levels of H_2S when fish are started as eggs, although there may be some apparent stimulation after shorter exposure at low levels. In fish started as adults, growth inhibition occurred at 0.0061 to 0.0071 mg/liter H_2S.

Food Consumption and Conversion

Consumption of live minnows by subadult bluegills at 28 days (BG-2) varied from 51.46 mg/g fish/day in the controls to 16.22 mg/g fish/day at 0.0105 mg/liter H_2S (Table 57). There was a substantial decrease in food intake only above 0.0062 mg/liter in 114 days. In a second test running for 97 days with prespawning adults, no difference was noted at levels up to 0.0078 mg/liter. Food conversion efficiency was decreased

Table 38. [illegible] Level (expressed as percentage)[a]

[illegible]	Exposure, days	H_2S, mg/liter				
		Control	0.0023	0.0042	0.0075	0.0092
BG-sp	28	42	32	34	25	18
[illegible]	37	39	31	30	23	14
	116	30	27	29	23	14
	200	39	27	27	16	14
	316	39	27	27	16	14
		Control	0.0012	0.0018	0.0034	0.0087
BG-sp	28	43	25	25	25	17
3&4	56	34	14	20	25	12
	86	34	12	18	25	11
	113	32	12	18	20	11
	130	32	12	18	20	11
		Control	0.0010	0.0013	0.0040	0.0073
BG-sp	28	21	17	13	18	10
5&6	93	2	2	3	2	1
		Control	0.0015	0.0031	0.0061	0.0064
BG-small	28	100	100	90	100	90
	362	100	100	90	100	90
	392	100	100	90	100	60
	420	100	100	90	100	0
	717	100	100	90	100	0
	826	100	100	90	70	0

Table 14 (continued). SURVIVAL OF BLUEGILLS WITH LONG-TERM EXPOSURE TO H_2S
(expressed as percentage)

Test	Exposure, days	H_2S, mg/liter: Control	0.0014	0.0023	0.0071	0.0090
BG-1	28	100	100	100	100	88
	224	100	100	100	100	88
	288	90	100	100	88	88
		Control	0.0010	0.0025	0.0062	0.0105
BG-2	28	100	100	100	100	56
	56	100	100	100	83	56
	200	100	100	100	83	56
		Control	0.0007	0.0014	0.0027	0.0078
BG-4	97	100	100	100	100	100

[a] Initial numbers for test BG-sp 1 & 2 were 200; BG-sp 3 & 4, 900; BG-sp 5 & 6, 800; BG-small, 10; BG-1, 10; BG-2, 6; BG-4, 10.

Table 36. GROWTH OF BLUEGILLS WITH LONG-TERM EXPOSURE TO H_2S
(expressed as mean weight in grams)

Test	Exposure, days	H_2S, mg/liter				
		Control	0.0031	0.0042	0.0075	0.0092
BG-sp	78	0.29	0.16	0.09	0.13	0.17
1&2	200	5.37	3.70	3.22	1.88	2.37
	316	10.70	7.12	8.70	5.29	4.36
		Control	0.0012	0.0018	0.0034	0.0087
BG-sp	86	0.17	0.21	0.20	0.15	0.11
3&4	113	0.45	0.56	0.54	0.31	0.39
		Control	0.0015	0.0031	0.0061	0.0064
BG-small	56	4.02	4.50	5.00	5.18	4.44
	112	13.62	10.50	12.03	11.98	14.04
	252	45.65	39.59	30.65	38.57	29.83
	392	50.81	46.34	34.37	43.35	31.76
	493	81.73	74.12	62.14	72.36	-
	603	84.09	76.59	68.05	75.41	-
	717	97.36	92.36	81.89	86.96	-
	826	99.91	98.71	90.35	63.05[a]	-
		Control	0.0014	0.0023	0.0071	0.0098
BG-1	196	182.43	155.83	163.03	132.38	157.58
	257	183.57	155.36	161.28	115.70	150.30
	288	187.04	175.18	189.50	120.10	110.05

Table [illegible] (continued). GROWTH OF BLUEGILLS WITH LONG-TERM EXPOSURE TO H_2S

(expressed as mean weight in grams)

Test	Exposure, days	H_2S, mg/liter				
		Control	0.0010	0.0025	0.0061[a]	0.0105
BG-2	0	46.85	41.46	43.90	35.88	48.53
	28	50.06	43.40	46.11	55.26	50.16
	56	51.51	42.06	48.18	55.40	45.66
	84	56.23	43.50	48.35	47.72	44.76
	114	71.05	49.50	60.23	58.90	50.28
	200	124.66	99.20	99.36	113.36	91.49
		Control	0.0007	0.0014	0.0027	0.0078
BG-4	0	75.90	84.00	78.20	76.10	76.70
	97	116.90	132.60	132.80	120.90	118.00

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[a] Value for concentration of 0.0061 dropped due to mortality of a large fish.

Table 57. [illegible] CONSUMPTION OF BLUEGILLS WITH LONG-TERM EXPOSURE TO H_2S

(expressed as milligrams food/gram fish/day)

Test	Exposure,[a] days		H_2S concentration, mg/liter				
			Control	0.0010	0.0025	0.0062	0.0103
BG-2	28		51.46	25.69	64.85	15.35	14.22
	56		73.65	24.01	58.58	13.30	15.82
	84		111.32	76.13	116.14	72.03	43.02
	114		149.47	104.47	166.99	150.40	94.91
		$\bar{\bar{x}}$ =	96.48	57.58	101.64	62.77	41.99
			Control	0.0007	0.0014	0.0027	0.0078
BG-4	97		35.89	31.95	34.31	32.39	36.26

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[a] Value expressed for each designation of exposure day is the mean consumption for the preceding 28-day period.

at all levels from 0.0010 to 0.0103 mg/liter H_2S in the first test but in the second, conversion efficiency did not appear to drop until a concentration of 0.0078 mg/liter was reached (Table 58). These conversion factors are consistent with the observed decrease in growth rate noted previously.

Reproduction

One chronic test (BG-small) running for 826 days failed to produce any spawning in treatments of 0.0015 to 0.0064 mg/liter H_2S (Table 59). Fish in the controls spawned with an average of 9,928 eggs/female and 130 eggs/g female. A second test (BG-4), started with adults and carried for 97 days in H_2S concentrations of 0.0007 to 0.0078 mg/liter, produced 153.5 eggs/g female in controls, 100.8 eggs/g female at 0.0007 mg/liter, and 51.1 eggs/g female at 0.0014 mg/liter H_2S. At 0.0027 and 0.0078 mg/liter H_2S there were no eggs deposited.

Failure to deposit eggs appeared directly related to behavior. In the two highest treatments there was no significant activity by the male over the nesting gravel and females did not lay eggs. In the lower concentrations activity was more restricted at 0.0014 than at 0.0007 mg/liter. Since no lower concentrations were attempted, the exact lower level of inhibition was not determined. The one third lower deposition of eggs/g female in this treatment than in the controls strongly suggests that any measurable level of H_2S will have some inhibitory effect.

Effect of H_2S Acclimation on Response to Anesthesia

During the progress of chronic experiments BG-sp 1 & 2 and 3 & 4, BG-small, and BG-1 and -2, fish were anesthetized periodically with MS:222 to permit weighing. In tests started with eggs and exposed for 288 days, the mean number of seconds required for anesthesia with increased H_2S concentration decreased from 89 in the control to 79 at 0.0092 mg/liter

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
BG-3	28	4.57	3.59	2.67	0.00	0.70
	56	1.36	0.00	2.64	0.68	0.00
	86	2.80	1.57	0.11	0.00	0.00
	114	5.58	4.41	4.72	4.98	4.37
		$\bar{x}$ = 3.57	2.39	2.54	1.42	1.27
		Control	0.0007	0.0044	0.0027	0.0078
BG-4	97	12.2	13.2	15.5	14.4	9.0

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[a] Calculated from increment in grams divided by grams of food consumed.

Table 59. SPAWNING SUCCESS OF BLUEGILLS WITH LONG-TERM EXPOSURE TO H_2S.

		H_2S concentration, mg/liter				
Test	**Item**	**Control**	**0.0015**	**0.0031**	**0.0061**	**0.0064**
BG-small (826 days)	Sex ratio(M:F)	3:3	2:3	5:2	4:3	-
	Total eggs	29,784	0	0	0	0
	Eggs/female	9,928	0	0	0	0
	Eggs/g female	130	0	0	0	0
		Control	**0.0007**	**0.0014**	**0.0027**	**0.0078**
BG-4 (97 days)	Sex ratio (M:F)	4:6	3:7	4:6	5:5	3:5
	Total eggs	104,967	89,315	36,935	0	0
	Eggs/female	17,562	12,795	6,157	0	0
	Eggs/g female	155.5	100.8	51.1	0	0

(Table [illegible]). [illegible] after 115 days of exposure, mean time to anesthesia decreased most markedly with 100 seconds in the control and 70 seconds at 0.0087 mg/liter H_2S. When fish were started as young-of-the-year and exposed for 826 days, the time for anesthesia was much shorter in all treatments through 378 days than in the controls but for the remainder of the test period there appeared to be no great difference. In tests started with bluegill adults (BG-1 and -2), exposures varied from 200 to 232 days and fish showed a substantial decrease in time to anesthesia in all H_2S treatments. The greatest difference was the reduction from 304 seconds in the control of one experiment to 188 seconds at a concentration of 0.0098 mg/liter H_2S.

Resistance to Other Stresses after Chronic Exposure

Experimental Design— Young-of-the-year bluegills (mean total length 3.2 cm) exposed 126 and 148 days to concentrations of H_2S ranging from 0.0004 to 0.0146 mg/liter were subjected to swimming endurance tests and to 5.5-9.2 mg/liter copper and to 0.075 mg/liter malathion. When fish were brought into the laboratory they were given prophylactic treatment with neomycin sulfate. After 3 days they were given a 3-day treatment with methylene blue. Fish were held at 21±1 C and fed brine shrimp and ground pork liver prior to chronic tests.

A series of 96-hr LC50 tests were conducted 28, 42, and 99 days after collection of fish in apparatus described by Adelman and Smith.[2] Test chambers were 6-liter acrylic cylinders 14.5 cm in diameter. Gaseous H_2S was employed and test concentrations were determined by analysis of water in centers of test chambers. Four levels of H_2S and one control were used and water flowed through chambers at a rate of 300 ml/min.

Long-term exposure tests were started on 100 fish 14 days after collection. They were placed in five tanks, each divided into three sections. Four test levels and one control were maintained and checked by analysis in each section. Fish in long-term tests were fed six times per day with various combinations of brine shrimp, ground beef liver, fresh ground minnows, and 1/32-inch Oregon moist pellets. A constant level of 7.8 pH was maintained with H_2SO_4 dispensed from a diluter similar to that used for the toxicant.

The swimming endurance was tested in a stainless steel oval raceway with a channel width of 8.5 cm, a depth of 15 cm, and a length of 218 cm. The channel included two straight sections each 50 cm long. Untreated laboratory water was maintained at a depth of 9 cm in the channel and the desired current was created with a stainless steel paddle wheel. The apparatus was similar to that described by Lemke and Mount.[16] Water velocity was regulated by a rheostat on the 1/8 h.p. motor drive of the paddle wheel. Fish were kept in position until they fell back by an electrical shocker placed downstream from their swimming location. One hundred twenty-volt AC current was used with the minimum amperage required to keep the fish away from the poles. One fiber glass screen was placed ahead of the poles to prevent fish from being injured by drifting between the electrodes and a second screen 22 cm upstream to form a swimming area 8.5 x 22 cm.

Test procedure was designed to minimize bias. A single fish was selected at random from an exposure tank, placed in a 400 ml beaker without removal from water, and transferred to the raceway swimming area. It rested in this position without electrical stimulation or water current for 30 min. Water temperature was held the same as that in the long-term exposure. In low speed tests after the 30-min rest period, current velocity was set at 8 cm/sec. After 5 min, current was increased to 11.2 cm/sec, and after 7 min to 15 cm/sec. At this time the electrical shocker was activated to prevent the fish from lying against the screen or holding its position by resting the caudal fin against the screen. At 9, 11, and 13 min the velocity was increased to 17.0, 19.5, and 22.5 cm/sec, respectively. The maximum velocity was maintained until the fish fell back against the screen. Electrical current was shut off, and

Table 60. TIME TO ANESTHESIA OF BLUEGILLS SUBJECTED TO LONG-TERM EXPOSURE TO H_2S (seconds)[a]

	MS:222	Exposure,	H_2S, mg/liter				
Test	mg/l	days	Control	0.0021	0.0042	0.0075	0.0092
BG-sp	80	86	73	79	82	73	72
1&2	80	116	79	77	70	79	80
	121	145	104	81	82	80	67
	130	170	86	93	99	105	100
	137	200	75	73	74	86	73
	137	230	106	74	74	107	68
	137	260	92	86	85	82	91
	137	288	98	89	81	74	78
			$\bar{x}$ = 89	82	81	86	79
			Control	0.0012	0.0018	0.0034	0.0087
BG-sp	80	86	110	112	98	93	87
3&4	80	113	95	94	94	60	53
			$\bar{x}$ = 102	103	96	76	70
			Control	0.0015	0.0031	0.0061	0.0064
BG-	137	56	357	156	148	101	105
small	137	112	443	124	122	128	122
	137	252	138	162	166	175	172
	137	392	101	89	85	82	69
	137	493	155	123	115	99	-
	137	578	196	192	159	141	-
	137	717	87	81	67	54	-
	137	826	90	106	121	104	-
			$\bar{x}$ = 196	129	123	110	117

Table [illegible] (continued). TIME TO ANESTHESIA OF BLUEGILLS SUBJECTED TO LONG-TERM EXPOSURE TO H_2S (seconds)[a]

Test	MS:222 mg/l	Exposure, days	Control	0.0016	0.0023	0.0072	0.0088
				H_2S, mg/liter			
BG-1	137	196	283	169	149	139	147
	137	252	326	219	186	180	228
			$\bar{\bar{x}}$ = 304	194	168	160	188
			Control	0.0010	0.0025	0.0062	0.0105
BG-2	137	84	194	170	159	128	102
	137	114	97	94	101	86	89
	137	200	124	96	113	123	128
			$\bar{\bar{x}}$ = 138	120	124	112	106

[a] Time notation based on complete anesthesia of all survivors in each concentration after specified days of exposure.

water movement stopped. After 5 min fish were anesthetized in MS:222, weighed, measured, and returned to an unoccupied section of their original test tank. One fish was taken from each tank successively until all fish from one section were tested. They were then returned to their original test section.

In high speed tests, fish were taken from section B of each long-term test and placed in the raceway. After a 30-min rest, water velocity was started at 8 cm/sec. After 5 min velocity was increased to 10.7 cm/sec, at 7 min to 17.5 cm/sec, and the electrical shocker activated. Subsequently at 9, 11, 13, 30, and 45 min, velocity was increased to 19.7, 22.5, 25.0, 27.0, and 28.0 cm/sec, respectively. After swimming failure of the fish, the procedure was the same as in low speed tests.

Chemical stress tests were carried out with apparatus similar to the proportional diluter used in long-term tests except that it was designed to dispense the same toxicant levels to all test chambers. The toxicant was introduced in a mixing box and then to a distribution box which split the volume into five equal portions for distribution to test chambers each of which was 50 x 26 x 30 cm. Water depth was 22 cm and cycle time was 2 min.

Reaction to Long-term and Short-term Exposure--The 96-hr LC50 tests were conducted on untreated fish 28, 42, and 99 days after bluegills were collected. Mean water conditions during the tests were 20 ± 0.1 C, 6.2 ± 0.4 mg/liter dissolved oxygen, 7.90 ± 0.05 pH meter reading, and 235 ± 0 mg/liter $CaCO_3$ total alkalinity. LC50 was calculated from five test levels and one control. Ninety-six-hour LC50 values for the three tests were 0.0325, 0.0320, and 0.0325 mg/liter H_2S. As noted above, each test tank in long-term tests was divided into three sections with fish placed in the first two. Since there was a reduction of H_2S levels between the first (A) and second (B) sections, analyses were run on each section. In series "A", H_2S concentrations extended from 0.0004 to 0.0146 mg/liter; in series "B", from 0.0004 to 0.0067 mg/liter (Table 61).

After 126 days of exposure in series "A", the concentrations had no effect on survival (Table 62) and growth in length appeared to be affected adversely only at the highest concentration (0.0146 mg/liter) where weight was less than two-thirds that attained in the control. Gill irrigation rate of treated fish was significantly increased over that in controls for all treatments and was 139% greater in the highest concentration.

After 148-day exposure in series "B", survival was not affected and growth was not adversely affected (Table 63). Gill irrigation rate determined visually increased in fish from all treatments and those from the highest (0.0067 mg/liter) had 42.5% greater irrigation rate than the controls.

Swimming endurance was adversely affected by chronic exposure to H_2S. In series "A", the low speed tests indicated that those from the lowest concentration (0.0004 mg/liter) of the series had slightly increased capability to endure swimming stress. Controls swam for 201 min before failure and fish from the highest treatment (0.0146 mg/liter) for 31 min or 84% shorter time than the controls (Table 62). Fish from series "B" in the lowest concentration had the least resistance (36% less than controls) (Table 63).

Resistance to Copper and Malathion--After fish finished swimming tests, they were returned to the treatment tanks and allowed to remain for 18 days before being subjected to tests with copper or malathion. Fish of the "A" series were used in copper tests. Copper in filtered samples was 3.8 mg/liter from all chambers except the one containing fish treated at 0.0015 mg/liter H_2S where concentration was 4.0 mg/liter (Table 64). Survival time varied from 13.5 hr in controls and lowest H_2S group to 52.5 hr in highest H_2S group (+288% of control survival time).

Table 61. TEST CONDITIONS OF SERIES A AND B DURING CHRONIC BLUEGILL TESTS[a]

		Series A			
$\bar{x}$ H_2S concentration (mg/l)	-	0.0004	0.0015	0.0048	0.0146
H_2S std. dev. (mg/l)	-	0.0006	0.0012	0.0020	0.0050
$\bar{x}$ pH	7.72	7.74	7.77	7.80	7.90
$\bar{x}$ temperature (C)	24.1	24.0	24.0	24.1	24.1
$\bar{x}$ dissolved O_2 (mg/l)	6.20	6.49	6.58	6.66	6.49
$\bar{x}$ total alkalinity (mg/l)	191	191	191	191	191
		Series B			
$\bar{x}$ H_2S concentration (mg/l)	-	0.0004	0.0007	0.0022	0.0067
H_2S std. dev. (mg/l)	-	0.0007	0.0006	0.0014	0.0032
$\bar{x}$ pH	7.72	7.73	7.77	7.79	7.91
$\bar{x}$ temperature (C)	23.7	23.5	23.6	23.7	23.7
$\bar{x}$ dissolved O_2 (mg/l)	5.87	6.21	6.18	6.13	6.01
$\bar{x}$ total alkalinity (mg/l)	191	191	191	191	191

[a] All values given are means of weekly or biweekly tests.

Table 62. EFFECT OF CHRONIC EXPOSURE OF BLUEGILLS TO VARIOUS LEVELS OF H_2S FOR 126 DAYS (SERIES A) ON GROWTH, GILL IRRIGATION RATE[a] AND SWIMMING ENDURANCE

Item	Series A (velocity 8.0 cm/sec)[b]				
H_2S concentration (mg/l)	-	0.0004	0.0015	0.0048	0.0146
Fraction of 96-hr LC50	-	1/81	1/22	1/7	1/2
Survival (%)	90	100	90	90	90
Mean total length (cm)[c]	5.68	5.21	4.78	5.51	4.54
Mean weight (g)	3.69	2.88	2.03	3.58	1.91
Mean gill irrigation rate (no./min)[a]	46	57	65	62	110
% of control	-	+24	+41	+35	+139
Swimming endurance					
Time to failure (min)	201	241	95	119	31
% of control	-	+20	-53	-41	-84

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[a] Gill irrigation check after 106 days exposure.

[b] Text explanation for speed change.

[c] Fish in all chambers had a mean length at start of chronic tests of 3.23 cm (range 2.70-4.80).

Table 63. EFFECT OF CHRONIC EXPOSURE OF BLUEGILLS TO VARIOUS LEVELS OF H_2S FOR 148 DAYS (SERIES B) ON GROWTH, GILL IRRIGATION RATE[a] AND SWIMMING ENDURANCE

Item	Series B (velocity 8-28 cm/sec)[b]				
H_2S concentration (mg/l)	0	0.0004	0.0007	0.0022	0.0067
Fraction of 96-hr LC50	-	1/81	1/46	1/15	1/5
Survival (%)	100	100	90	100	100
Mean total length (cm)[c]	5.42	5.00	5.22	5.40	5.14
Mean weight (g)	2.86	2.16	2.59	3.18	3.42
Mean gill irrigation rate (no./min)[a]	52	71	73	73	74
% of control	-	+36.5	+40.5	+40.5	+42.5
Swimming endurance					
Time to failure (min)	28	18	21	19	22
% of control	-	-36	-25	-32	-21

[a] Gill irrigation check after 106 days exposure.
[b] Text explanation for speed change.
[c] Fish in all chambers had a mean length at start of chronic tests of 3.23 cm (range 2.70-4.80).

Table 64. RESISTANCE OF BLUEGILLS WITH CHRONIC TREATMENT OF H_2S TO SUBSEQUENT EXPOSURE OF COPPER SULFATE (AS CU) (SERIES A) AND MALATHION (SERIES B)[a]

Item			Series A		
H_2S concentration (mg/1)[a]	0	0.0004	0.0015	0.0048	0.0146
Copper (mg/1) - unfiltered	5.5	7.5	9.2	8.2	7.0
filtered	3.8	3.8	4.0	3.8	3.8
Temperature (C)	24.3	24.1	24.3	24.5	24.0
Dissolved O_2 (mg/1)	6.65	6.61	6.63	6.63	6.58
Survival time (hr)	13.5	13.5	12.5	24.0	52.5
% control	-	0	-7	+78	+288
			Series B		
H_2S concentration (mg/1)	0	0.0004	0.0007	0.0022	0.0067
Malathion (mg/1)	0.075	0.075	0.075	0.075	0.075
Temperature (C)	24.0	24.0	24.1	23.9	23.9
Dissolved O_2 (mg/1)	7.79	8.05	8.05	7.87	8.02
Survival time (hr)	72.5	94.0	75.5	72.5	72.7

[a] Total alkalinity in Series A was 212 mg/1 and in Series B, 216 mg/1; pH in Series A was 7.5 and in Series B, 7.6.

The "B" series fish were tested with malathion at a concentration of 0.075 mg/liter. Controls and highest treatment survived for 72.5 hr. Fish conditioned at the lowest H_2S treatment (0.0004 mg/liter) had a longer survival time than controls or highest treatment (94.0 hr) (Table 64).

SUMMARY

The most sensitive stage of bluegill development as measured by LC50 is the swim-up fry with the median lethal threshold concentration of 0.0084 mg/liter H_2S at 39 days after hatching. The most resistant stages are juvenile and adult with mean 96-hr LC50 of 0.0316 and 0.0297 mg/liter H_2S, respectively. In chronic exposures the gross response most sensitive to sublethal levels of H_2S is spawning which was inhibited at 0.0007 mg/liter and eliminated at 0.0027 mg/liter H_2S. Growth rate gave no consistent response to the toxicant in most tests until more than 100 days of exposure. In tests started with fry measurable response was obtained in 78 days.

The gross effects of long-term exposure to H_2S were reduced growth in the highest concentration and progressively increased gill irrigation rate with increased concentrations of H_2S. This increase in irrigation rate suggests decreased efficiency in the oxygen uptake or transport. In the lower speed swimming stress tests given the series "A" fish, pretreatment at the lowest concentration appeared to increase endurance; but at treatment levels of 0.0015 mg/liter H_2S and higher, fish had progressively less endurance. Fish treated at the highest level (0.0146 mg/liter) were much more easily stunned by electrical shock at the time of failure than those treated at lower levels. In the high speed tests all fish showed much less resistance to the swimming stress but differences among treatments and controls was less marked than in low speed tests at comparable treatment levels.

Resistance to copper was increased by exposure to H_2S but resistance to malathion was not affected except in the lowest concentration. Since copper affects oxygen uptake by gills and H_2S-oxygen relationships in the blood, the increased irrigation rate induced by exposure to higher levels of H_2S may account for the higher tolerance to copper by treated fish. At the cellular level, H_2S combines with metallic elements (Goodman and Gillman[17]). Whether this reaction influenced resistance to copper in the present experiments was not determined.

From the data developed in this study, it is apparent that slow speed swimming tests will reveal adverse effects of long-term exposure to H_2S on bluegills except at the highest concentrations better than the other gross indicators of changes except where spawning behavior is involved. It is also apparent that extended exposure to H_2S levels of 0.0015 mg/liter and greater reduces the physical capability of the fish.

SECTION VIII

WALLEYE

(*Stizostedion vitreum vitreum* (Mitchill))

Toxicity of H_2S to walleye was determined by (1) four acute tests on juveniles and (2) two chronic tests started as juveniles. Egg and fry data were taken from a previous project reported by Smith and Oseid[18].

ACUTE TESTS

Experimental Design

Acute tests to determine LC50 values for H_2S were made on juvenile walleyes collected from Sand Shore Lake near Bethel, Minnesota. Fish were planted in the lake as fry and later removed by seine as juveniles. Fish were held in the laboratory prior to testing at 12 C and were fed live fathead minnows in excess of consumption once a day. Immediately after arrival at the laboratory fish were treated for 3 days with 20 mg/liter neomycin. They were subjected to a routine of 12 hr of light and 12 hr of darkness. Fish were raised gradually to test temperature and held at test temperature for 7 days prior to start of acute tests. Flow-through apparatus described by Adelman and Smith[2] was used in all tests. The test chambers were of glass-silicone construction with Acute tests 1-3 having 38-liter capacity and test 4, 25-liter capacity. Flow rate through each chamber was 30 ml/min. Each test consisted of one control and five H_2S concentrations. The same light regime used for holding was maintained during the test. Water samples for H_2S analyses were made three times daily during each test and for temperature, oxygen, and total alkalinity once per day. Fish were not fed during the 96-hr test.

Acute Toxicity

The four acute tests (Table 65) were run at 14.8-16.1 C and at O_2 levels of 5.9-6.8 mg/liter. The 96-hr LC50 varied from 0.0166 to 0.0214 with a mean of 0.0193 mg/liter H_2S.

Acute toxicity of H_2S to eggs and fry was determined prior to the present project and reported by Smith and Oseid.[18] In three tests on eggs at 15 C they found that the 96-hr LC50 varied from 0.074 to 0.087 mg/liter H_2S. In four tests at 12 C, 96-hr LC50 ranged from 0.052 to 0.066 mg/liter H_2S. LC50 at hatch in periods from 7 to 19 days ranged from 0.022 to 0.066 mg/liter H_2S with temperature varying from 12-15 C and O_2 from 3 to 6 mg/liter. The higher values were obtained with O_2 levels at 4 and 3 mg/liter. Tests at longer periods were from start of incubation and short tests from partial incubation until hatch was complete. Fry survival at all H_2S levels tested was substantially below the control and at concentrations of 0.040 mg/liter and higher was greatly reduced. At the higher levels the percentage of fry deformity ran from 50% to 84% with the probability of survival of deformed fry negligible.

CHRONIC TESTS

Experimental Design

Two chronic tests started with walleye juveniles collected from Sand Shore Lake were conducted for 225 and 231 days. Two additional tests were carried on but abandoned after disease problems destroyed their validity. Test apparatus was that described by Brungs and Mount[4] which provided a flow-through of 500 ml/min. Test chambers were insulated fiber glass tanks 200 x 53 x 53 cm filled with water to contain 340 liters. Each test included one control and four H_2S concentrations

Table 65. ACUTE TEST CONDITIONS AND LC50 VALUES FOR WALLEYE JUVENILES TESTED IN H_2S

Test	Days from collection to start of test	Mean length[b], mm	Mean test conditions[a]		LC50, mg/l H_2S			
			Temp., C	O_2, mg/l	24 hr	48 hr	72 hr	96 hr
1	76	90	15.9	5.9	0.0413	0.0314	0.0196	0.0183
2	89	89	16.1	6.0	-	0.0274	0.0229	0.0214
3	76	92	14.8	6.8	-	0.0178	0.0174	0.0166
4	143	100	15.0	6.2	-	-	-	0.0210
								$\bar{x}$ = 0.0193

[a] pH 7.8-8.0 in separate tests.
[b] 5 fish per chamber.

(Table 66). Tests 1 and 2 started with 10 hr of light and 14 hr of darkness and were gradually adjusted to 16 hr of light and 8 hr of darkness by the time the test was terminated.

Fish were fed during the test with live minnows in excess of consumption. The number of dead minnows removed from the tank were subtracted from the number of live minnows introduced to give daily consumption of minnows. Daily minnow consumption was converted to grams on the basis of mean weekly weight of minnows fed. Fish were held prior to start of test and acclimated in the same manner as those used in acute tests. Chronic test 1 concentrations of H_2S ranged from 0.0013 to 0.0051 mg/liter and in test 2 from 0.0031 to 0.118 mg/liter (Table 66).

Survival

Survival in test 1 appeared to be affected adversely in 225 days at 0.0051 mg/liter H_2S and in test 2 at 0.0118 mg/liter H_2S in 231 days (Table 67). The large variation in concentration maintained during the test as indicated by standard deviation throws question on the exact concentration which could be assumed to affect survival. Misfunction of dispensing apparatus accounted for the variability.

Growth Rate

Growth as indicated by weight (g) and length (mm) was not significantly affected at any of the concentrations of H_2S tested (Tables 68 and 69). Consumption of minnows (mg/g fish/day) by juveniles which were exposed to H_2S is shown in Table 70. Efficiency of food utilization based on increased fish weight compared to food intake in grams was higher in H_2S concentration than in controls and especially at the lower concentrations (Table 71). This relationship may have resulted from reduced general activity noted in the tanks treated with H_2S.

Fish were measured and weighed after immobilization with MS:222. The time in seconds to immobility for fish in both tests was substantially

Table 66. PHYSICAL AND CHEMICAL CONDITIONS IN CHRONIC TESTS ON WALLEYES

			Test 1		
$\bar{x}$ H_2S concentration (mg/l)	Control	0.0013	0.0020	0.0031	0.0051
H_2S std. dev. (mg/l)	-	0.0008	0.0020	0.0025	0.0042
$\bar{x}$ pH	7.59	7.62	7.65	7.67	7.71
$\bar{x}$ temperature (C)	17.6	17.8	17.8	17.8	18.0
$\bar{x}$ dissolved O_2 (mg/l)	8.1	8.1	8.1	8.1	8.1
$\bar{x}$ total alkalinity (mg/l)	235	235	235	235	235
			Test 2		
$\bar{x}$ H_2S concentration (mg/l)	Control	0.0031	0.0053	0.0057	0.0118
H_2S std. dev. (mg/l)	-	0.0027	0.0081	0.0070	0.0105
$\bar{x}$ pH	7.51	7.62	7.60	7.60	7.61
$\bar{x}$ temperature (C)	19.6	19.6	19.7	19.7	19.6
$\bar{x}$ dissolved O_2 (mg/l)	8.3	8.3	8.3	8.3	8.3
$\bar{x}$ total alkalinity (mg/l)	182	182	182	182	182

Table 67. SURVIVAL OF WALLEYE DURING CHRONIC EXPOSURE TO H_2S (expressed as percentage)[a]

Test	Exposure, days	H_2S, mg/l				
		Control	0.0013	0.0020	0.0031	0.0051
1	28	100	100	100	100	90
	56	100	100	100	100	82
	112	100	100	100	100	82
	140	100	100	100	100	73
	225	100	100	100	100	73
		Control	0.0031	0.0053	0.0057	0.0118
2	28	100	100	100	100	93
	197	100	100	100	100	93
	231	100	100	100	100	87

[a] 10 fish used per tank. Some fish lost from causes unrelated to treatment.

Table 68. MEAN WEIGHT OF JUVENILE WALLEYE AFTER EXPOSURE TO H_2S^a (grams)

Test	Exposure, days	H_2S, mg/l				
		Control	0.0013	0.0020	0.0031	0.0051
1	28	7.49	8.35	8.07	7.67	7.52
	56	14.28	14.83	15.34	14.55	13.64
	84	22.72	24.53	25.06	23.44	20.94
	112	34.62	40.03	34.50	35.27	21.81
	140	47.57	53.70	50.01	45.68	43.18
	168	56.80	61.37	55.58	54.81	55.61
	196	59.76	72.46	64.12	61.89	65.85
	225	61.60	88.36	74.93	70.74	72.79
		Control	0.0031	0.0053	0.0057	0.0118
2	0	5.11	5.11	5.56	4.76	5.14
	28	9.59	11.09	11.14	10.44	10.39
	57	15.51	17.11	16.18	15.83	15.01
	85	21.39	22.70	19.53	21.01	19.03
	113	24.31	25.34	21.78	25.16	22.69
	141	26.77	25.94	23.53	27.41	24.24
	169	29.95	28.75	26.19	30.53	25.38
	197	41.02	33.73	35.23	50.13	29.38
	231	60.81	66.17	59.23	71.68	59.41

[a] 10 fish per tank.

Table 69. MEAN LENGTH OF JUVENILE WALLEYE AFTER EXPOSURE TO H_2S[a] (millimeters)

	Exposure,	H_2S, mg/l				
Test	**days**	**Control**	**0.0013**	**0.0020**	**0.0031**	**0.0051**
1	28	9.64	9.71	9.69	9.36	9.51
	56	12.19	11.12	12.40	12.17	11.90
	84	14.59	14.53	14.88	14.22	13.65
	112	16.41	16.81	16.71	16.30	16.30
	140	18.40	18.48	18.24	17.90	17.57
	168	19.20	19.79	19.20	19.05	19.10
	196	19.83	20.68	20.03	19.94	20.32
	225	20.27	22.50	21.37	20.80	21.23
		Control	**0.0031**	**0.0053**	**0.0057**	**0.0118**
2	0	9.54	9.47	9.68	9.34	9.38
	28	11.27	11.59	11.54	11.23	11.26
	57	12.97	13.31	13.24	13.13	12.99
	85	14.67	14.55	14.36	14.60	14.32
	113	15.48	14.42	14.96	15.40	15.00
	141	15.96	15.63	15.40	16.00	15.60
	169	16.62	15.78	15.88	16.51	16.12
	197	17.04	17.00	17.37	19.38	17.32
	231	20.24	20.50	19.78	21.36	19.85

[a] 10 fish per tank.

Table 70. MINNOW CONSUMPTION OF JUVENILE WALLEYES EXPOSED TO H_2S[a]
(milligrams/grams fish/day)

Test	Exposure, Exposure, days	H2S concentration, mg/liter: Control	0.0013	0.0020	0.0031	0.0051
1	29-56	88.24	85.42	82.91	89.11	75.61
	57-84	100.54	106.20	100.00	96.32	97.17
	85-112	69.59	69.08	64.47	73.91	84.66
	113-140	58.64	53.99	59.87	61.76	75.38
	141-168	29.32	31.46	29.55	35.03	48.18
	169-196	25.74	37.36	34.92	34.96	42.15
	197-225	23.73	42.28	42.58	39.81	43.42
		$\bar{x}$ = 56.54	60.83	59.19	61.56	66.65
		Control	0.0031	0.0053	0.0057	0.0118
2	0-28	179.59	182.72	156.89	177.63	135.31
	29-57	36.65	33.33	30.75	33.49	32.28
	58-85	66.12	53.27	50.95	59.17	62.28
	86-113	48.14	42.05	53.73	52.86	55.13
	114-141	31.32	30.81	36.63	31.58	33.67
	142-169	42.67	39.14	44.65	34.86	46.35
	170-197	54.11	87.39	77.82	50.83	61.36
	198-231	78.55	103.90	85.12	63.88	97.07
		$\bar{x}$ = 67.14	71.58	67.07	63.04	65.43

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[a] 10 fish per tank.

Table 71. EFFICIENCY OF FOOD CONVERSION BY WALLEYE EXPOSED TO H_2S[a]
(expressed as percentage)

Test	Exposure, days	H_2S, mg/l				
		Control	0.0013	0.0020	0.0031	0.0051
1	28- 56	25.1	23.4	26.7	24.7	27.2
	57- 84	16.2	16.6	17.2	17.3	15.6
	85-112	21.3	24.8	23.2	19.5	21.5
	113-140	19.2	19.3	17.7	14.8	16.6
	141-168	21.5	15.1	12.7	18.5	18.6
	169-196	7.0	15.8	14.6	12.4	14.3
	197-225	4.6	16.7	13.0	12.0	8.2
		Control	0.0031	0.0053	0.0057	0.0118
2	0- 28	12.1	14.4	15.2	15.0	17.8
	29- 57	44.4	44.2	41.4	42.2	38.8
	57- 85	17.2	18.8	17.0	13.1	14.4
	86-113	9.5	9.3	12.1	7.2	10.5
	114-141	11.0	2.7	9.7	7.5	7.0
	142-169	9.4	9.4	11.0	8.6	3.5
	170-197	20.6	6.5	34.1	13.5	8.5
	198-231	17.7	22.3	19.1	22.0	24.9

[a] 10 fish per tank; efficiency calculated from increment in grams divided by food intake in grams.

shortened at all levels of H_2S treatment (Table 72).

SUMMARY

Acute toxicity as measured by 96-hr LC50 had a mean among four tests on juveniles of 0.0193 mg/liter H_2S. In the chronic tests a potentially adverse effect at all treatment levels was observed through the response of fish to anesthesia. On the basis of survival, a no-effect level on juveniles and older fish is 0.0030 mg/liter H_2S. High variation in test concentrations make effect data less accurate.

Table 72. TIME TO IMMOBILITY IN MS:222 OF WALLEYE JUVENILES[a] EXPOSED TO H_2S (seconds)

Test	Exposure, days	H_2S, mg/l				
		Control	0.0013	0.0020	0.0031	0.0051
1	28	145	71	84	93	114
	56	100	91	90	140	183
	84	100	145	207	349	101
	112	130	190	157	172	192
	140	87	92	89	95	87
	168	94	126	124	123	107
	196	142	117	99	109	88
	225	144	83	83	87	77
		Control	0.0031	0.0053	0.0057	0.0118
2	28	60	65	51	55	55
	57	73	65	62	52	46
	85	66	63	55	49	44
	113	82	66	59	46	42
	141	79	64	61	56	46
	169	61	57	54	48	43
	197	73	57	66	53	49

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[a] 10 fish per tank; time based on immobility of all fish from each treatment.

SECTION IX

BROOK TROUT

(*Salvelinus fontinalis* (Mitchill))

Toxic effects of H_2S to brook trout were determined by (1) 96-hr LC50 and LTC tests on eggs, sac fry, feeding fry, and juveniles, (2) chronic exposure of adults and juveniles, and (3) swimming endurance tests on fish previously exposed to low levels of H_2S.

ACUTE TESTS

Experimental Design

Six bioassays were conducted on eggs with a proportional diluter similar to that designed by Mount and Brungs[5] and Brungs and Mount.[4] Sodium sulfide was used as a source of H_2S. The egg chamber consisted of acrylic plastic cylinders 18 cm long with an inner diameter of 4.5 cm. Eggs were supported on a Nitex screen attached to the bottom of the cylinder and were shielded from light. Water flowed through a 25-liter tank with the cylinders attached to the outlet. Flow was 600 ml/min. Eggs used in the tests were stripped from trout held in the laboratory and from a trout hatchery near Osceola, Wisconsin. Four tests were made on sac fry from eggs incubated in fresh water. Apparatus was the same as that used for eggs. Four bioassays were run on feeding fry ranging in length from 2.1-2.8 cm. Fry were fed five times daily on ground Glencoe trout food and Oregon moist and acclimated to test temperature for 7 days prior to test. Test chambers were glass (19.5 x 20.5 x 21 cm). Ninety-five percent replacement of water in the test chambers was made in 30 min. Sixteen tests on juvenile trout were run in glass chambers (50.8 x 25.4 x 25.4 cm) with the outlet adjusted to maintain a volume of 25 liters. The same diluters used for eggs and fry were used for juveniles. Ninety-five percent replacement of water was made in 3 hr 20 min. Fish were not fed during first 96 hr of test but received food thereafter.

Tests for H_2S were done from water at outlet five times per day. Fluorescent light was applied to all tests for 12 of each 24 hr. In each test of eggs, fry, and juveniles five levels of toxicant and one control were used.

Acute Toxicity

Eyed Eggs--Eyed eggs were used in acute tests. H_2S concentrations ranged from 0.0189 to 0.1026 mg/liter. No 96-hr LC50 was calculated. Mean lethal threshold concentrations (LTC) were 0.0761 mg/liter H_2S at 8.5 C in 240 and 312 hr and 0.0501 mg/liter H_2S at 13.5 C in 192 and 216 hr (Table 73). At 9.4 C and 8.9 C, 50% mortality had not occurred after 460 and 108 hr, respectively, because treatment levels were not sufficiently high.

Sac Fry--Sac fry 48 hr after hatch were tested over a range of H_2S concentrations of 0.0077-0.0370 mg/liter. Mean 96-hr LC50 and lethal threshold concentration in 240 hr at 8.5 C were 0.0210 and 0.0160 mg/liter H_2S, respectively (Table 74). Mean 96-hr LC50 and lethal threshold concentrations at 13.5 C were 0.0148 and 0.0120 mg/liter H_2S, respectively.

Feeding Fry--Feeding fry were tested over a range of H_2S concentrations of 0.0140-0.0255 mg/liter. Mean LTC's for 8.5 and 13.5 C were 0.022 and 0.186 mg/liter H_2S, respectively (Table 75).

Juveniles--Prior to testing juveniles were acclimated at the test tem-

Table 73. THRESHOLD TOXICITY (LTC) OF H_2S TO BROOK TROUT EGGS[a]

Test	Mean tempera- ture, C	Mean pH	H_2S concentrations, mg/liter					LTC, mg/liter, H_2S	Hours
1	9.4	7.71	0.0325 (0.0087)	0.0407 (0.0084)	0.0461 (0.0167)	0.0538 (0.0121)	-	-	460
2	8.9	7.76	0.0586 (0.0149)	0.0728 (0.0306)	0.0968 (0.0171)	0.1062 (0.0383)	-	-	108
3	8.5	7.67	0.0290 (0.0044)	0.0391 (0.0067)	0.0551 (0.0097)	0.0725 (0.0122)	0.0915 (0.0170)	0.0783	312
4	8.5	7.71	0.0277 (0.0049)	0.0364 (0.0032)	0.0488 (0.0030)	0.0632 (0.0047)	0.0805 (0.0101)	0.0738	240
5	13.5	7.70	0.0189 (0.0102)	0.0351 (0.0146)	0.0450 (0.0155)	0.0583 (0.0213)	-	0.0516	192
6	13.5	7.72	0.0199 (0.0076)	0.0349 (0.0132)	0.0416 (0.0143)	0.0541 (0.0157)	-	0.0485	216

[a] Standard deviations in parentheses; eyed eggs used for tests.

Table 74. 96-HOUR LC50 AND LTC VALUES OF H_2S TO BROOK TROUT SAC FRY

Test	Mean tempera- ture, C	Mean pH	H_2S concentrations[a], mg/liter					96-hour LC50, mg/liter H_2S	LTC, mg/liter H_2S	Hours
1	8.5	7.69	0.0126	0.0160	0.0216	0.0291	0.0370	0.0206	0.0161	240
			(0.0012)	(0.0030)	(0.0036)	(0.0036)	(0.0064)			
2	13.5	7.67	0.0077	0.0098	0.0128	0.0151	0.0225	0.0138	0.0117	240
			(0.0016)	(0.0012)	(0.0022)	(0.0026)	(0.0047)			
3	8.5	7.69	0.0121	0.0162	0.0214	0.0279	0.0363	0.0214	0.0160	240
			(0.0013)	(0.0019)	(0.0028)	(0.0019)	(0.0024)			
4	13.5	7.67	0.0084	0.0120	0.0149	0.0176	0.0245	0.0158	0.0124	240
			(0.0012)	(0.0022)	(0.0013)	(0.0013)	(0.0009)			

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[a] Standard deviations in parentheses.

Table 75. 96-HOUR LC50 AND LTC VALUES OF H_2S TO BROOK TROUT FEEDING FRY[a]

Test	Mean temperature, C	Mean pH	H_2S concentrations, mg/liter					96-hour LC50, mg/liter H_2S	LTC, mg/liter H_2S	Hours
1	8.5	7.69	0.0180 (0.0020)	0.0210 (0.0020)	0.0220 (0.0022)	0.0235 (0.0027)	0.0255 (0.0029)	0.0234	0.0220	240
2	13.5	7.74	0.0150 (0.0025)	0.0171 (0.0029)	0.0200 (0.0029)	0.0220 (0.0035)	0.0250 (0.0037)	0.0215	0.0186	216
3	13.5	7.71	0.0140 (0.0014)	0.0171 (0.0019)	0.0189 (0.0016)	0.0206 (0.0020)	0.0240 (0.0022)	0.0217	0.0187	216
4	8.5	7.73	0.0185 (0.0018)	0.0218 (0.0019)	0.0230 (0.0022)	0.0242 (0.0027)	- -	0.0232	0.0226	240

[a] Standard deviations in parentheses; fry 95 days after fertilization of eggs.

peratures for 7 days except at 18.5 and 21 C when 14-day periods were used. Values were obtained for 96-hr LC50 and threshold (LTC) after 144 to 244 hr at 8.5, 11, 13.5, 16.0, 18.5, and 21.0 C. Sixteen tests were conducted over a period from April to October and mean size increased from 5 to 16 cm in successive bioassays.

Fish were tested over a range of H_2S concentrations of 0.0053 to 0.0346 mg/liter. Mean 96-hr LC50's showed a range of 0.0266 mg/liter H_2S at 8.5 C to 0.0178 mg/liter H_2S at 21 C. Mean lethal threshold concentrations had a range of 0.0197 mg/liter at 8.5 C to 0.0078 mg/liter H_2S at 21 C (Table 76, Figure 6).

The percentage changes in mean 96-hr LC50 and threshold values for each 2.5 C increment from 8 to 21 C were determined (Table 77). Mean 96-hr LC50 values decreased 33.1% with increasing temperature and mean threshold values decreased 60.4% from 8 to 21 C (Figure 7).

Mean times in hours to threshold LC50 decreased with increasing temperature from 8.5 to 13.5 C, but began to increase with increasing temperature between 13.5 and 16 C (Table 77). At the lowest temperature tested (8.5 C) deaths occurred slowly and evenly throughout the test. Intermediate temperatures (11, 13.5, and 16 C) produced death less slowly but evenly over a shorter time period. At higher temperatures (18.5 and 21 C) most deaths occurred quickly, but some occurred unevenly over a long time period. In the bioassays at higher temperatures most mortality occurred quickly, but some continued sporadically over extended time periods (144 and 288 hr; Table 76) similar in duration to the low temperature bioassays.

Influence of Temperature on Various Stages--To determine the effects of temperature on acute toxicity to all life history stages except adults (eyed egg, sac fry, feeding fry, and juvenile) and the relative resistance of various stages, a comparison was made of the H_2S threshold

Table 76. 96-HOUR LC50 AND LTC VALUES OF H_2S TO BROOK TROUT JUVENILES

Test	Mean temperature, C	Mean pH	H_2S concentrations[a], mg/liter					96-hour LC50, mg/liter H_2S	LTC, mg/liter H_2S	Hours
1	8.1	7.72	0.0165 (0.0034)	0.0178 (0.0039)	0.0200 (0.0041)	0.0226 (0.0042)	0.0251 (0.0049)	0.0245	0.0187	264
2	8.1	7.71	0.0166 (0.0030)	0.0189 (0.0033)	0.0208 (0.0048)	0.0235 (0.0043)	0.0245 (0.0058)	0.0256	0.0191	288
3	8.5	7.69	0.0148 (0.0020)	0.0181 (0.0027)	0.0213 (0.0049)	0.0254 (0.0044)	0.0346 (0.0033)	0.0297	0.0212	288
4	11.0	7.69	0.0107 (0.0016)	0.0140 (0.0033)	0.0175 (0.0034)	0.0214 (0.0025)	0.0264 (0.0038)	0.0228	0.0171	216
5	11.1	7.70	0.0089 (0.0014)	0.0141 (0.0019)	0.0170 (0.0023)	0.0210 (0.0046)	0.0258 (0.0041)	0.0233	0.0184	216
6	13.5	7.68	0.0109 (0.0019)	0.0136 (0.0017)	0.0172 (0.0018)	0.0211 (0.0016)	0.0252 (0.0027)	0.0219	0.0153	264
7	13.6	7.78	0.0116 (0.0142)	0.0196 (0.0217)	0.0224 (0.0244)	0.0233 (0.0301)	0.0271 (0.0302)	0.0174	-	144
8	13.4	7.85	0.0107 (0.0050)	0.0143 (0.0056)	0.0156 (0.0081)	0.0183 (0.0086)	- -	0.0156	0.0156	144

Table 76 (continued). 96-HOUR LC50 AND LTC VALUES OF H_2S TO BROOK TROUT JUVENILES

Test	Mean temperature, C	Mean pH	H_2S concentrations[a], mg/liter					96-hour LC50, mg/liter H_2S	LTC, mg/liter H_2S	Hours
9	15.9	7.73	0.0125 (0.0028)	0.0141 (0.0020)	0.0149 (0.0021)	0.0171 (0.0028)	0.0188 (0.0046)	0.0163	0.0141	192
10	15.9	7.72	0.0124 (0.0021)	0.0149 (0.0028)	0.0168 (0.0030)	0.0183 (0.0027)	0.0189 (0.0022)	0.0173	0.0146	192
11	16.0	7.67	0.0097 (0.0024)	0.0120 (0.0016)	0.0133 (0.0021)	0.0155 (0.0022)	0.0171 (0.0029)	-	0.0129	240
12	16.0	7.67	0.0094 (0.0018)	0.0117 (0.0024)	0.0129 (0.0020)	0.0153 (0.0022)	0.0178 (0.0021)	-	0.0137	240
13	18.5	7.67	0.0116 (0.0020)	0.0123 (0.0023)	0.0138 (0.0022)	0.0156 (0.0018)	0.0173 (0.0025)	0.0168	-	-
14	18.5	7.67	0.0110 (0.0015)	0.0119 (0.0023)	0.0140 (0.0018)	0.0154 (0.0019)	0.0173 (0.0022)	0.0168	-	-
15	21.0	7.68	0.0063 (0.0016)	0.0083 (0.0013)	0.0111 (0.0021)	0.0146 (0.0025)	0.0183 (0.0034)	0.0177	0.0078	240
16	21.0	7.70	0.0053 (0.0013)	0.0074 (0.0015)	0.0103 (0.0015)	0.0155 (0.0018)	0.0207 (0.0018)	0.0179	0.0078	288

[a] Standard deviations in parentheses.

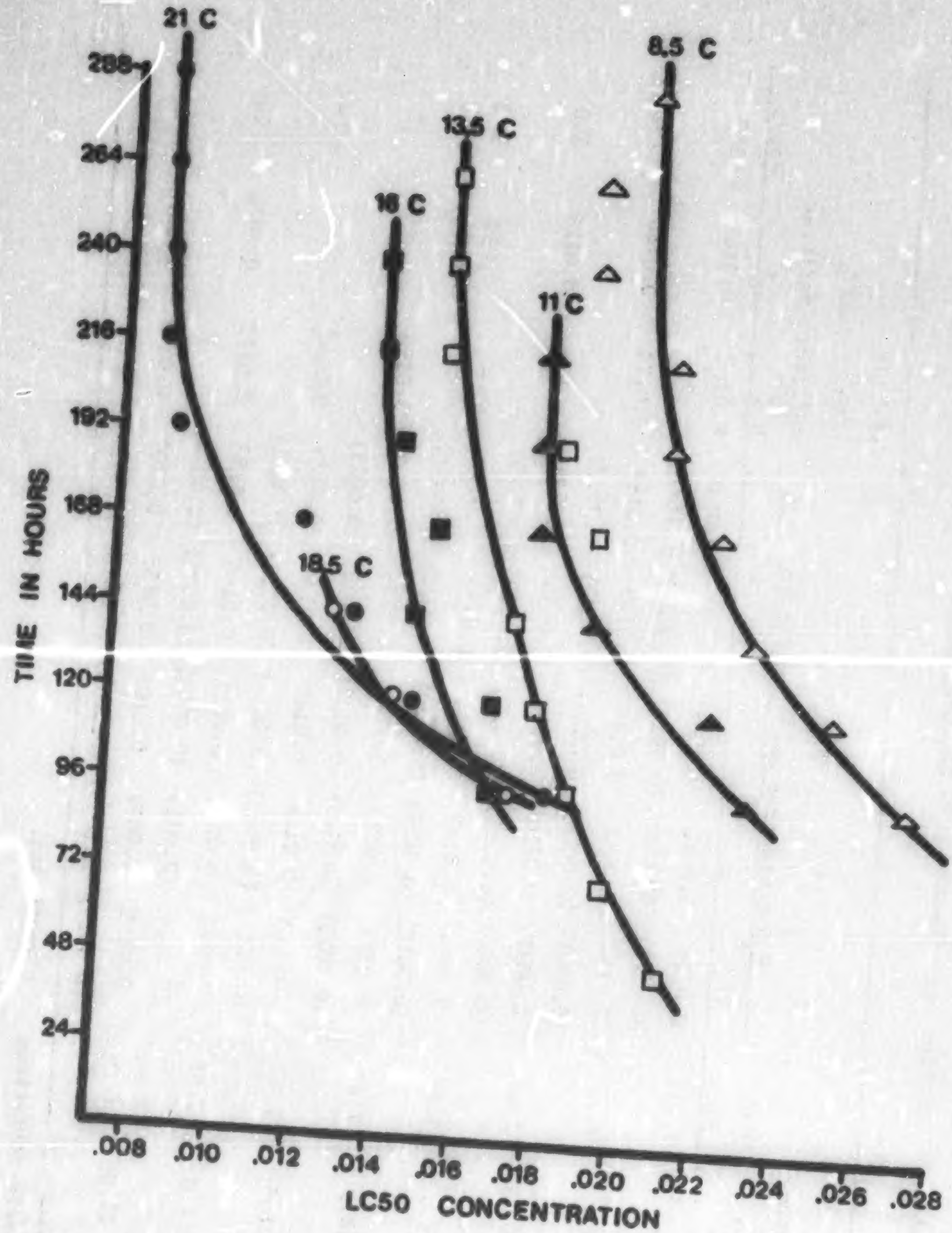

Figure 6. Toxicity curves for brook trout juveniles at 2.5 C intervals from 8.5 to 21.0 C (expressed as mg/liter H_2S).

Table 77. A COMPARISON OF 96-HOUR LC50 AND LTC VALUES OF H_2S FOR 2.5 C INCREMENTS FROM 8.5 TO 21.0 C IN BROOK TROUT JUVENILES[a]

Number of tests	Temperature, C	96-hr LC50			LTC			Mean time, hr
		Mean, mg/l	Mean difference, mg/l	Mean difference, %	Mean, mg/l	Mean difference, mg/l	Mean difference, %	
3	8.5	0.0266			0.0197			280
			-0.0036	-13.6		-0.0019	-9.6	
2	11.0	0.0230			0.0178			216
			-0.0047	-20.5		-0.0023	-12.9	
3	13.5	0.0183			0.0155			204
			-0.0015	-8.2		-0.0017	-11.0	
4	16.0	0.0168			0.0138			216
			0	0		-	-	
2	18.5	0.0168			-			-
			+0.0010	+6.0		-	-	
1	21.0	0.0178			0.0078			252
15	8.5-21.0		-0.0088	-33.1		-0.0119	-60.4	234

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[a] Mean differences are between LC50 or LTC at successive temperatures.

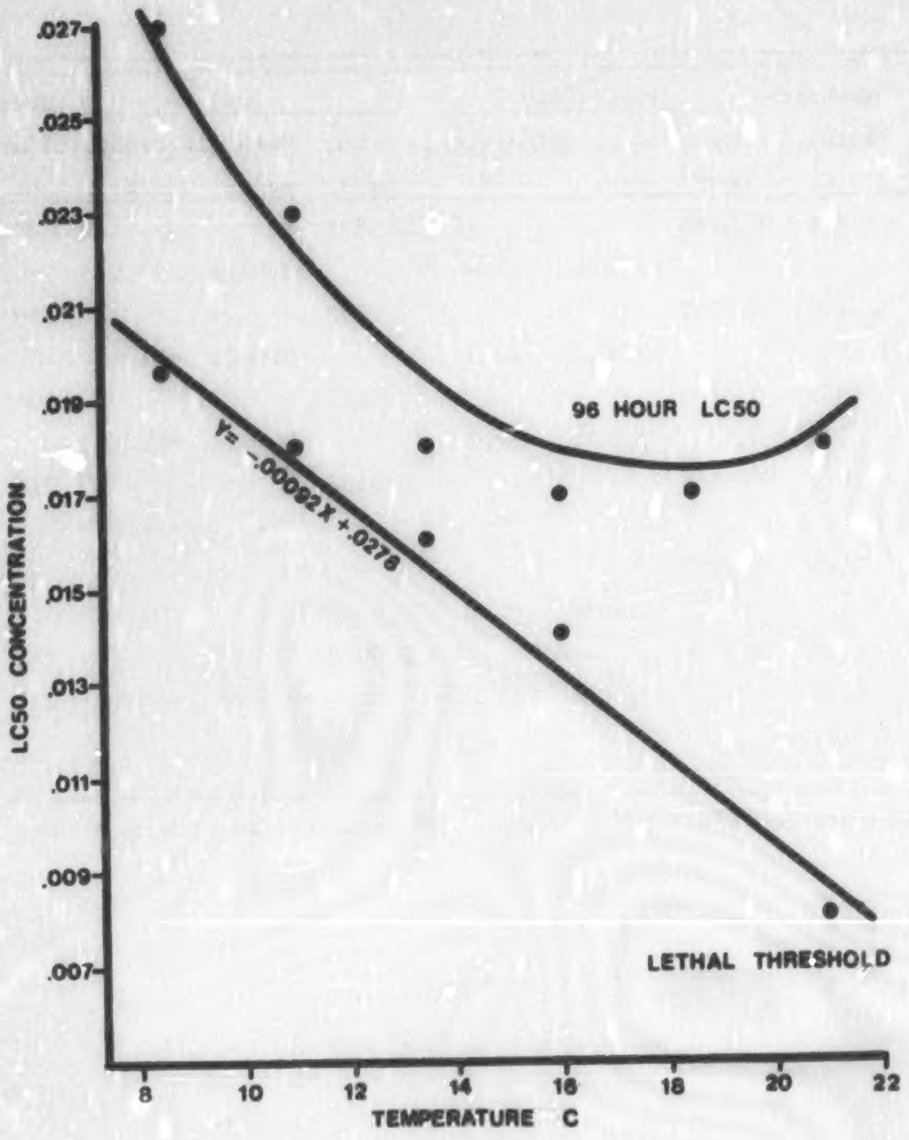

Figure 7. Mean 96-hr LC50's (quadratic) and lethal threshold concentrations (linear) for brook trout at 8.5 to 21.0 C (expressed as mg/liter H_2S; 96-hr LC50 fitted by eye).

values (Table 78). At 8.5 and 13.5 C the most resistant stage to H_2S was the egg, followed by feeding fry, juvenile, and sac fry in that order (Table 79). Eggs were more than three times as tolerant as juveniles. The influence of temperature on the various stages was greatest in eggs where a 34.2% decrease in resistance occurred with an increase in temperature from 8.5 to 13.5 C. The least difference was in feeding fry with a decrease of 16.1%. The resistance of eyed eggs to H_2S toxicity is probably due to the low respiration rates of incubating trout eggs. Sac fry susceptibility to H_2S toxicity is due to a variety of reasons (Olson and Marking[19]): large surface area capable of absorption of H_2S, no well developed detoxifying system, and a high metabolic rate.

CHRONIC TESTS

Three chronic bioassays were run on juveniles and adult brook trout to determine the effects of low concentrations of H_2S on growth of juveniles, reproduction by adults, and swimming endurance of juveniles.

Experimental Design

Two chronic tests running for 72 and 120 days were conducted on brook trout to determine effects of H_2S on growth. Fish in the 72-day test were started as 23-day-old fry with a mean weight of 0.09 g. The 120-day test was started with 166-day-old juveniles with a mean weight of 5 g. Proportional diluters previously described were used to supply water and toxicant to the test chambers. In the 72-day experiment, 25 fry were placed in each of 10 aquaria (51 x 24 x 20 cm). After 30 days fish from the aquaria were placed in 10 fiber glass tanks (164 x 56 x 52 cm), each divided into two equal parts containing 28 liters. The experiment consisted of eight H_2S levels ranging from 0.0015 to 0.0142 mg/liter and two controls (Table 80). Mean pH was 7.72 (7.59-7.95), temperature 9.0 C (9.0-9.1), and oxygen 7.9 mg/liter (7.8-8.0). Fish were fed _ad libitum_ Oregon moist and Glencoe pellets two times per day. Periodic weighings of fish were made _en masse_ from each tank. They were

Table 78. PERCENTAGE DIFFERENCES IN MEAN LTC VALUES OF H_2S BETWEEN 8.5 AND 13.5 C FOR EACH LIFE HISTORY STAGE OF BROOK TROUT

Stage	Temperature, C	Mean LTC, mg/l H_2S	% change	Mean LTC time, hr
Eyed egg	8.5	0.0760	-34.2	276
	13.5	0.0500		204
Sac fry	8.5	0.0160	-25.0	240
	13.5	0.0120		240
Feeding fry	8.5	0.0223	-16.1	244
	13.5	0.0187		216
Juvenile	8.5	0.0197	-21.8	280
	13.5	0.0154		204

Table 79. PERCENTAGE DIFFERENCES IN MEAN LTC VALUES OF H_2S BETWEEN EACH SUCCESSIVE LIFE HISTORY STAGE OF BROOK TROUT AT 8.5 AND 13.5 C

	8.5 C		13.5 C	
Stage	Mean LTC, mg/liter H_2S	% change	Mean LTC, mg/liter H_2S	% change
Eyed egg	0.0760		0.0500	
		-78.9		-76.0
Sac fry	0.0160		0.0120	
		+30.9		+55.8
Feeding fry	0.0223		0.0187	
		-11.7		-17.6
Juvenile	0.0197		0.0154	

transferred to water in a beaker and differential weight between water and water containing fish was determined.

In the 120-day test 20 juvenile fish were placed in each of the 10 fiber glass tanks described above with eight levels of toxicant ranging from 0.0015 to 0.0130 mg/liter and two controls (Table 81). Fish were fed *ad libitum* Oregon moist and Glencoe pellets two times per day. Mean pH was 7.69 (7.30-8.15), temperature 13.2 C (13.0-13.3), and oxygen 7.8 mg/liter (7.6-8.3). Weighing was done *en masse* as in the 72-day test. Flow-through rate in both tests was 600 ml/min. H_2S was tested from water taken out of the central portion of the tank five times per week.

Growth Rate

In the 72-day test changes in growth rate were not progressive with increased concentrations of H_2S but at 0.0032 mg/liter and higher some reduction occurred (Table 80). After 60 days there was a marked decrease in growth rate at 0.0090 mg/liter and higher levels. At the conclusion of the test fish kept in 0.0140 mg/liter H_2S were 45% smaller than the mean of the controls. Since no statistical treatment on the basis of individual fish was made, exact significance of the effect of fish variability on apparent growth difference cannot be made. On the assumption that difference less than 10% may not have been significant, it is believed that no adverse effect was shown at concentrations less than 0.0066 mg/liter H_2S.

The 120-day test started with 166-day-old juveniles showed depressed growth rate at all treatment levels (Table 81). After 60 days, treatments with H_2S concentration of 0.0090 mg/liter and higher had mean weights 16 to 24% lower than the controls. After 90 days, growth was 6% below controls at 0.0067 mg/liter and 22% below at 0.0090 mg/liter. After 120 days, growth was 14% below control at 0.0067 mg/liter and 53% below at 0.0125 mg/liter. The major growth reduction appeared to occur between 0.0067 and 0.0090 mg/liter H_2S.

Table 80. WEIGHT OF JUVENILE BROOK TROUT AT SUCCEEDING INTERVALS IN VARIOUS H_2S CONCENTRATIONS AT 9 C IN 72 DAYS[a]

(grams)

				H_2S concentration, mg/liter[b]							
				0.0015	0.0032	0.0051	0.0066	0.0090	0.0119	0.0140	0.0142
Day	Control$_1$	Control$_2$	Mean	(0.0005)	(0.0011)	(0.0014)	(0.0018)	(0.0027)	(0.0018)	(0.0015)	(0.0032)
0	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
30	0.25	0.32	0.28	0.28	0.20	0.21	0.29	0.23	0.24	0.16	0.21
				[0]	[-29]	[-25]	[+4]	[-18]	[-14]	[-43]	[-25]
60	1.01	0.91	0.96	0.92	0.92	1.02	0.96	0.86	0.71	0.55	0.61
				[-4]	[-4]	[+6]	[0]	[-10]	[-26]	[-33]	[-36]
72	1.28	1.22	1.25	1.24	1.18	1.15	1.21	1.07	0.86	0.72	0.74
				[-1]	[-6]	[-8]	[-3]	[-14]	[-31]	[-42]	[-41]
Total[c]	1.19	1.13	1.16	1.15	1.09	1.06	1.12	0.98	0.77	0.63	0.65
				[0]	[-6]	[-8]	[-3]	[-15]	[-33]	[-45]	[-43]

[a] Percentage comparison of weight in various H_2S concentrations with the mean weight of control groups in brackets.

[b] Standard deviations in parentheses.

[c] Increment in 72 days.

Table 81. WEIGHT OF JUVENILE BROOK TROUT IN VARIOUS H_2S CONCENTRATIONS AT 13 C IN 120 DAYS[a]

(grams)

				H_2S concentration, mg/liter[b]							
Day	$Control_1$	$Control_2$	Mean	0.0015 (0.0015)	0.0034 (0.0015)	0.0050 (0.0013)	0.0067 (0.0017)	0.0090 (0.0030)	0.0097 (0.0024)	0.0125 (0.0033)	0.0130 (0.0029)
0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
30	7.7	7.5	7.6	7.2	7.4	7.6	7.2	6.4	7.0	6.3	6.5
				[-5]	[-3]	[0]	[-5]	[-16]	[-8]	[-17]	[-14]
60	12.3	12.4	12.4	11.5	11.6	11.5	12.0	9.4	11.0	10.4	10.4
				[-7]	[-6]	[-7]	[-3]	[-24]	[-24]	[-16]	[-16]
90	19.2	18.9	19.0	18.8	17.8	17.0	17.8	14.8	16.6	13.5	15.0
				[-1]	[-6]	[-11]	[-6]	[-22]	[-13]	[-29]	[-21]
120	33.2	33.3	33.2	30.2	26.4	30.3	29.2	23.2	26.5	18.2	-[c]
				[-9]	[-20]	[-9]	[-12]	[-30]	[-20]	[-45]	-
Total[d]	28.2	28.3	28.2	25.2	21.4	25.3	24.2	18.2	21.5	13.2	-
				[-11]	[-24]	[-10]	[-14]	[-35]	[-24]	[-53]	-

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[a] Percentage comparison of weight in various H_2S concentrations with the mean weight of control groups in brackets.

[b] Standard deviations in parentheses.

[c] This tank not weighed due to poor survival (35%) of smaller fish.

[d] Increment in 120 days.

Reproduction

A third chronic test was started with adult brook trout to determine the influence of prespawning exposure to spawning success. The experimental design was the same used for the previous chronic tests except that spawning boxes were provided shortly prior to anticipated first spawning. The spawning boxes consisted of marine plywood with two fiber glass screen windows. Box size was 51 x 58 x 16 cm. Temperatures were gradually reduced from 13.8 C in August to 9 C in October. A nominal temperature of 9 C was then maintained throughout the spawning and subsequent incubation period. One hundred trout, each weighing approximately 500 g, were distributed between 10 tanks on August 8 with five males and five females in each tank. Size grading at the hatchery where fish were secured provided an extremely uniform stock. To insure sex identification during bioassay, adipose fins on male trout were clipped. Test concentrations varied from 0.0055 to 0.0128 mg/liter H_2S (Table 82). Equipment malfunction eliminated three treatments at 0.0010, 0.0030, and 0.0050 mg/liter. Mean pH was maintained at 7.78 (7.40-8.23), temperature at 9.1 C (6.2-13.8), and mean oxygen at 7.5 mg/liter. H_2S concentrations were checked three times per week.

Fifty-five days after start of treatment on October 5 trout in the control first spawned and continued thereafter until October 18 (Table 82). In most cases spawning took place in the early morning hours and eggs were removed from spawning boxes within 12 hr of deposition. A spawning was defined as deposition of 50 or more eggs in the spawning box. Two days after spawning started in the control, spawning started in a concentration of 0.0055 mg/liter H_2S, the lowest treatment. Seven spawnings were made over the following 27-day period until October 30. Four females spawned between October 4 and 10 but there was no further spawning until October 27 and 30, when the fifth female spawned twice (Table 83). At a concentration of 0.0079 mg/liter H_2S spawning started on October 5. By October 15 five of the six total spawnings had taken place. On October 26 the final spawning occurred. With a treatment of

Table 82. BROOK TROUT REPRODUCTION DATA IN VARIOUS H_2S CONCENTRATIONS

Tank	H_2S concentration, mg/liter[a]	Spawnings	Dates	Number of eggs	% alive[b]	Number of fish M	Number of fish F	Mean eggs/ spawning	Mean eggs/ female
10	control	10	10/2-10/18	3,797	76.8	4	5	380	759
3	0.0055(0.0021)	7	10/4-10/30	2,222	73.6	5	5	317	444
9	0.0079(0.0024)	6	10/5-10/26	1,731	74.1	5	5	288	346
6	0.0109(0.0026)	1	10/31	328	70.4	5	5	328	66
7	0.0121(0.0030)	3	10/20-10/30	1,254	52.4	4	4	418	314
8	0.0128(0.0043)	3	10/6-10/10	1.396	72.7	4	5	465	279

[a] Standard deviations in parentheses.

[b] Percentage of eggs alive immediately after spawning (within 12 hours).

Table 83. SPAWNING DATES OF BROOK TROUT DURING THE MONTH OF OCTOBER

	H_2S concentration, mg/liter				
Control	0.0055	0.0079	0.0109	0.0121	0.0128
2	4	5	31	20	6
2	4	7		27	8
4	6	8		30	10
4	7	14			
6	9	15			
9	27	26			
10	30				
12					
16					
18					

0.0109 mg/liter H_2S a single spawning took place on October 31, 29 days after initial spawning in the control. Throughout much of the period preceding egg deposition in this concentration spawning behavior was greatly depressed. Occasionally a female lingered in the spawning box but males showed no interest. Not until October 24 was any significant activity noted, when a female fanned out the spawning box. The initial spawning at 0.0121 mg/liter H_2S occurred on October 20. Two more spawnings followed on October 27 and 30. In this concentration spawning activity was also greatly depressed throughout the greater part of the month. On October 19 the first fanning of the spawning box was noted. In each case spawning in this tank followed a 24-hr period of accidentally decreased H_2S concentration. It will be noted from the standard deviation ±0.0030 mg/liter that variability of H_2S concentration was quite large. In the highest concentration (0.0128 mg/liter H_2S) trout were being held at near the lethal concentration. Any increase over that target level caused the trout great stress. There were large variations in the standard deviation of concentration in this tank and the three spawnings which did occur on October 6, 8, and 10 followed a 3-day period of decreased H_2S levels. The concentration was reduced to prevent mortality following a slug of higher concentration. It was evident that fish at all H_2S concentrations tested were capable of spawning throughout October if the stress were temporarily relieved. However, at 0.0109 mg/liter H_2S and higher fish were prevented from spawning (that is, they showed little or no interest in spawning activity) unless short periods of reduced H_2S concentration occurred. All spawning activity was stopped at 0.0109, 0.0121, and 0.0128 mg/liter H_2S when target levels were restored. It is believed that consistently maintained levels of 0.0109 mg/liter and higher would totally prevent spawning. The test was continued until January but no spawnings occurred after October 30.

In the control there was an average of two spawnings per female with the first spawning usually producing the largest number of eggs. Although the number of spawnings by individual females is not known, in no case was the average of two spawnings per female obtained in the H_2S

concentrations. The percentage of eggs alive immediately after spawning in the treatments differed little from that in the control (Table 82).

The number of eggs deposited per female decreased with increased concentration of H_2S (Table 82). The total number of spawnings, the total number of eggs spawned per tank, and the number of eggs per female decreased rapidly with increased concentration. The control had 759 eggs per female and the H_2S treatments varied from 444 in the lowest concentration to 279 in the highest concentration. At the lower levels where spawning activity was not greatly depressed the effective spawning period was much extended.

Viability of Eggs--After each spawning the eggs were removed from the spawning box and a random sample placed in an incubation basket in the treatment where eggs were spawned and also in the control tank. Eggs from successive spawnings were randomly distributed to the same baskets as the first spawning. Samples of eggs deposited in the control tank were placed in baskets in the control and also in each of the treatment levels (Table 84). Baskets were attached to oscillating arms which maintained water movement over the eggs. Hatching success of eggs spawned and incubated in the control tank was 77%. The best hatch of eggs spawned in any H_2S treatment and incubated in the control tank was 30% (Table 84). Eggs spawned in the control tank and incubated in various H_2S treatments had much better hatching success than eggs spawned and incubated in the same H_2S treatments. This difference may be caused by water absorption immediately following egg deposition for a 30-hr period. Eggs spawned and incubated in individual treatments may absorb a lethal or near-lethal dosage of H_2S. Eggs spawned in a treatment tank but incubated in a control tank had decreased mortality. Eggs spawned in the control tank and incubated in the various treatment tanks had survival levels only slightly less than those spawned and incubated in controls.

Table 84. PERCENTAGE HATCH OF BROOK TROUT EGGS, DAYS TO HATCH, AND LENGTH OF FRY AT HATCH

Eggs spawned in control and incubated in H_2S treatments (Group A); eggs spawned in each treatment and incubated in same treatment (Group B); and eggs spawned in each treatment and incubated in control tank (Group C).

Group	H_2S concentration, mg/liter[a]	Number of eggs	Number hatched	% hatch	Mean fry length, cm	Days to hatch
A	0.0055 (0.0021)	150	59	39.3	2.0	54.5
	0.0079 (0.0024)	200	104	52.0	2.0	56.2
	0.0109 (0.0026)	150	79	52.7	2.0	55.8
	0.0121 (0.0030)	200	123	61.5	2.0	59.2
	0.0128 (0.0043)	70	49	70.0	2.0	56.4
B	0.0055 (0.0021)	225	87	38.7	2.0	49.8
	0.0079 (0.0024)	250	13	5.2	1.9	51.8
	0.0109 (0.0026)	100	0[b]	0	-	-
	0.0121 (0.0030)	250	0[b]	0	-	-
	0.0128 (0.0043)	150	37	24.7	2.1	61.4
C	Control	100	77	77.0	2.0	57.8
	0.0055 (0.0021)	192	54	28.1	2.0	57.6
	0.0079 (0.0024)	225	67	29.8	2.1	57.2
	0.0109 (0.0026)	100	0	0	-	-
	0.0121 (0.0030)	150	28	18.7	2.0	55.4
	0.0128 (0.0043)	150	42	28.0	2.1	56.4

[a] Standard deviation in parentheses. Treatment concentrations are those continued through prespawning, spawning, and incubation periods.

[b] Loss due to temporary malfunction of apparatus.

Examination of hatching data indicates that eggs spawned in a treatment and incubated in a treatment have poor resistance down to 0.0053 mg/liter H_2S (39% survival). Survival of control spawned and incubated eggs (77%) at 9 C appears to be a good measure of spawning and hatching success under experimental conditions and is consistent with the observed production hatchery practice. The length of fry at hatch (Table 84) does not appear to be affected by exposure of eggs or adults prior to spawning. Days to mean hatch were not significantly affected by H_2S concentrations in the two lowest concentrations except that eggs hatched slightly faster than controls. In the higher levels eggs hatched somewhat slower than controls.

Swimming Endurance

After 45 and 120 days of exposure in the 120-day chronic growth bioassay previously described, 10 fish from each control and six treatment levels (70 fish) were tested for swimming endurance in a raceway filled with untreated laboratory water at 13 C. The raceway consisted of a revolving acrylic hood, 128 cm in diameter, covering a circular channel. The revolving hood which had alternate strips of black markings gave the test fish an illusion of water movement in the raceway and caused it to swim around the circular channel. An electrical barrier of probes attached to the hood forced the fish to swim at the revolving hood velocity. When the fish could no longer keep pace with the hood, it fell against or through the probes and was stunned. Two simulated water velocities, 50.3 cm/sec and 66.4 cm/sec, were used after 45 days and 120 days of exposure, respectively. The time in seconds to failure was noted for each H_2S concentration. After the 45-day tests the two highest H_2S concentrations were increased from mean values of 0.0114 and 0.0119 mg/liter to 0.0125 and 0.0130 mg/liter (Table 85). A consistent decrease in endurance times with an increase in H_2S concentration was observed. A multiple range test at 99% confidence level showed significant differences between performance of control fish and fish from treatments of 0.0090 mg/liter H_2S and greater after 45 and 120 days of chronic

Table 85. SWIMMING ENDURANCE OF BROOK TROUT JUVENILES AFTER 45 AND 120 DAYS OF EXPOSURE TO H_2S AT 13 C[a]

(time in seconds)

Exposure,	H_2S concentration, mg/liter						
45 days	Control	0.0037	0.0070	0.0090	0.0097	0.0114	0.0119[b]
Mean	315	304	303	228	232	182	194
Range	221-456	168-616	134-610	168-288	188-302	100-261	94-415
120 days[c]	Control	0.0034	0.0067	0.0090	0.0097	0.0125	0.0130[d]
Mean	376	358	345	214	182	182	-
Range	280-545	240-435	225-530	125-230	135-230	75-300	-

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[a] Raceway hood velocity set at 50.3 cm/sec (45 days) and 66.4 cm/sec (120 days); 10 fish per treatment level.

[b] Approximately 35% of the fish in this tank died and most were smaller than the average size fish in the tank.

[c] After 45 days of exposure to lower levels (0.0037-0.0119 mg/liter) concentrations were raised above starting levels in the two highest treatments.

[d] After 120 days poor fish survival (35%) and poor fish condition prevented testing.

exposure (Table 85). Direct examination of the fish during endurance tests often showed that fish larger and smaller than average swam for shorter times than the average within any given treatment, particularly at the higher concentrations. Endurance time, however, did not differ greatly with size of fish within a given concentration.

SUMMARY

Acute test were run on brook trout eggs, sac fry, feeding fry, and juveniles to determine 96-hr LC50's and LTC values up to 460 hr. These tests showed that at 13.5 C the most resistant stage was the eyed egg (LTC 0.0501 mg/liter H_2S), followed by feeding fry (LTC 0.0186 mg/liter), juvenile (LTC 0.0155 mg/liter), and sac fry (LTC 0.0120 mg/liter) in that order. The greatest influence of temperature on resistance to H_2S was noted in the egg stage and the least difference in the feeding fry. With increase in temperature from 8.5 to 21.0 C there was a steady decrease in resistance of juveniles to H_2S. A slightly greater than twofold decrease occurred in LTC and about 35% decrease in 96-hr LC50. Chronic tests running up to 120 days indicated that growth rate at all H_2S concentrations was lower than in the control. Reproduction was reduced in all H_2S concentrations tested. The number of spawnings per female was less and the number of eggs per spawning was reduced from that in controls. At all levels of H_2S there was a definite inhibition of spawning success and at 0.0079 mg/liter and higher reduction was to less than half the controls. The effect of H_2S appeared to be primarily suppression in spawning behavior. Swimming endurance was significantly reduced in all fish exposed for periods of 45 to 120 days at 0.0090 mg/liter H_2S and higher concentrations tested. At the highest level (0.0125 mg/liter) after 120 days of exposure, swimming endurance expressed in seconds to exhaustion was approximately half that of the controls. The data show that levels for protecting all life history stages and functions will be less than 0.0055 mg/liter H_2S.

SECTION X

RAINBOW TROUT

(Salmo gairdneri Richardson)

Toxicity of H_2S to rainbow trout was tested by (1) acute tests on sperm viability, success of fertilization, eggs, fry, and juveniles and (2) chronic tests to determine survival, growth rate in H_2S and in a combination of H_2S and phenol.

ACUTE TESTS

Experimental Design

The acute tests of H_2S toxicity on rainbow trout include one on sperm viability, one on success of fertilization, one on eggs, one on fry, and two on juveniles (Table 86). The test on sperm viability (Acute 1) was conducted by stripping males taken from 13 C water and held for 2 days at 10 C. One male was used for each series with no pooling of sperm. Sperm were distributed at random in each series. One drop of sperm was placed in control or H_2S concentration water contained in a Syracuse watch glass. A drop of mixture was immediately placed on a slide and duration of motility determined under a microscope (200x) equipped with heat-absorbing filter over the light source. The fertilization experiment (Acute 2) was done by stripping male and female fish treated as in the previous test. Ten cc of unfertilized eggs were placed in water from control or H_2S treatment and 2 drops of milt were added and mixture stirred gently. The mixture was held for 3 min and eggs were then transferred to control water and incubated for 10 days

Table 86. SOURCE OF RAINBOW TROUT AND STAGE OF FISH AT START OF TESTS WITH H_2S

Test	Stage at start	Date obtained	Source
Acute			
1	Sperm	2/11/71	State Hatchery, Lanesboro, Minn.
2	Fertilization	2/11/71	" " " "
3	Egg	2/11/71	" " " "
4	Fry	2/11/71	" " " "
5	Juvenile	23/3/70	White's Trout Farm, Paradise, Utah
6	Juvenile	2/11/71	State Hatchery, Lanesboro, Minn.
Chronic			
1	Fry and juvenile	23/3/70	White's Trout Farm, Paradise, Utah
2	Eyed egg	5/10/71	Ennis National Fish Hatchery, Ennis, Montana
3	Newly fertilized eggs	3/11/71	State Hatchery, Lanesboro, Minn.
4	Eyed egg	3/11/71	" " " "
5	Eyed egg	16/12/71	White's Trout Farm, Paradise, Utah
6	Eyed egg	16/12/71	" " " " "
7	Juvenile	16/12/71	" " " " "

in a Heath-Techna hatching battery with laboratory water at 12.2 C (Table 87). At 10 days the percentage of fertile eggs was determined.

The acute egg test (Acute 3) was started with eggs stripped from adults as described for the previous test. Eggs were fertilized in control water and after water hardening for 2 hr were placed in test tanks at various H_2S concentrations. Twelve H_2S concentrations and three controls were maintained through egg hatch at 12.6 C. Test chambers were glass-silicone (20 x 20 x 20 cm) with a water volume of 6 liters. Eggs rested on the bottom of the chambers. The apparatus for dispensing toxicant was a modified Mount and Brungs[4] diluter which provided a flow-through rate of 200 ml/min. Test chambers were illuminated at 12 hr per day with 40-watt incandescent bulbs.

The fry test (Acute 4) was started with fry hatched from eggs incubated in control water as in previous tests. At hatching the fry were placed in twelve H_2S concentrations and three controls. Light was controlled as in the egg test.

Two juvenile tests (Acute 5 and 6) were started with fish 45 and 54 mm long, respectively. Flow-through apparatus as described by Adelman and Smith[2] was used in both tests. Test chambers were glass-silicone type (50 x 25 x 20 cm) containing 20 liters of water. Flow-through rate was 300 ml/min. Fish were reared from eggs at 12 C and were fed Glencoe fry granules up to the time of testing. No feeding was done during the 96 hr of the tests. Feeding with Glencoe granules was resumed in test 5 which ran more than 96 hr. Illumination was by fluorescent tubes.

In all acute tests samples of water were taken daily from the center of each test chamber and tested for temperature, pH, dissolved O_2, and total alkalinity. Water samples for H_2S determinations were taken twice daily from the center of each test chamber. Day length in acute tests 5 and 6 was 12 hr.

Table 87. ACUTE TEST CONDITIONS AND LC50 VALUES FOR RAINBOW TROUT TESTED IN H_2S[a]

Test	Stage	Days from collection to start of test	Number fish per chamber	Mean length, mm	Mean test conditions		LC50, mg/1 H_2S			
					Temp., C	O_2, mg/1	48 hr	72 hr	96 hr	LTC (days)
1	Sperm	2	—	—	12.2	9.4	-	-	-	-
2	Fertili-zation	3	—	—	12.2	9.4	-	-	-	-
3	Egg	1	150	—	12.6	9.4	-	-	-	0.0154 (29)
4	Fry	31	30	22	13.1	7.8	-	-	-	0.0056 (20)
5	Juvenile	91	10	45	12.3	8.4	-	-	0.0130	0.0087 (17)
6	Juvenile	113	10	54	15.1	6.4	0.0150	0.0130	0.0125	-

[a] pH 7.7 (average of meter readings).

Sperm Motility

Newly ejected sperm were placed in H_2S concentrations ranging from 0.0022 to 0.0589 mg/liter in two replications each from a single male (Table 88). In two replications of three control water tests (six determinations), the mean survival time in seconds as judged by discernible motion under magnification varied from 68.5 to 97.5. In the various H_2S concentrations survival time varied between 82.0 and 92.5 sec. In all but one of the H_2S treatments survival was slightly longer than the mean of all controls.

Egg Fertility

Fertility of eggs inseminated in various H_2S concentrations from 0.0096 to 0.0570 mg/liter and incubated for 10 days in control water at 12.2 C showed no trends with increasing concentrations (Table 89).

Egg Survival

Eggs fertilized in control water and incubated in various H_2S concentrations from 0.0045 to 0.0598 mg/liter had decreasing survival to hatch with increase in H_2S concentration (Table 90). From 0.0045 to 0.0236 mg/liter H_2S survival of eggs was better than in controls. Above that level survival was very low, with none hatching at 0.0598 mg/liter. Length of fry at hatch did not vary significantly from the controls except at 0.0469 mg/liter H_2S. The calculated LC50 of eggs at 29 days when hatch was completed was 0.0154 mg/liter H_2S.

Fry Survival

Fry were hatched from eggs incubated in control water and subjected to various concentrations of H_2S from 0.0027 to 0.0068 mg/liter for 20 days (Table 91). In controls with three replications length of fry after 20 days was 26.0 mm. At all H_2S concentrations growth was slightly slower than in controls and markedly so at 0.0068 mg/liter H_2S. Fry did not survive in concentrations greater than 0.0068 mg/liter.

Table 88. DURATION OF RAINBOW TROUT SPERM VIABILITY IN H_2S IN ACUTE TEST 1

H_2S concentration, mg/l	Duration of motility, seconds		
	1	2	Mean
Control	69.0	68.0	68.5
Control	84.0	111.0	97.5
Control	73.0	74.0	73.5
0.0022	103.0	83.0	93.0
0.0031	88.0	67.0	77.5
0.0083	84.0	78.0	81.0
0.0086	88.0	97.0	92.5
0.0105	90.0	89.0	89.5
0.0164	98.0	94.0	96.0
0.0214	79.0	95.0	87.0
0.0246	95.0	79.0	87.0
0.0420	76.0	92.0	84.0
0.0436	89.0	92.0	90.5
0.0484	77.0	87.0	82.0
0.0589	79.0	100.0	89.5

Table 89. FERTILITY OF RAINBOW TROUT EGGS INSEMINATED IN H_2S

H_2S concentration, mg/l	Fertilization,[a] %
Control	84
0.0096	75
0.0113	78
0.0144	90
0.0158	85
0.0451	87
0.0570	94

[a] Determined after 10 days by presence of an embryo.

Table 90. LENGTH OF RAINBOW TROUT FRY AT HATCH AND PERCENTAGE SURVIVAL OF EGGS INCUBATED IN H_2S AT 12.6 C

H_2S concentration, mg/l	Mean length, mm	Egg survival to hatch, %	Time to first hatch,[a] days
Control	13.8	46[b]	26
Control	13.8	27[b]	26
Control	13.5	17[b]	26
0.0045	14.1	66	26
0.0067	13.7	87	26
0.0105	14.0	16	26
0.0116	13.6	87	26
0.0117	13.4	70	26
0.0236	12.9	55	26
0.0279	13.1	7	26
0.0293	13.2	8	27
0.0469	11.5	4	26
0.0598	--	0	--

[a] All eggs dead or hatched in 29 days.

[b] Survival in controls was low from excessive turbulence; therefore survival in other treatments was not corrected to controls.

Table 91. LC50 VALUES FOR RAINBOW TROUT FRY AND LENGTH OF FRY AT VARIOUS H_2S CONCENTRATIONS IN 20 DAYS OF EXPOSURE (ACUTE TEST 6)

LC50		Fry length (20 days)	
Days	mg/l H_2S	H_2S, mg/l	Length, mm
5	0.0127	Control	26.0
7	0.0097	Control	26.0
10	0.0079	Control	26.0
12	0.0075	0.0027	25.5
13	0.0076	0.0032	25.5
17	0.0057	0.0037	25.5
20	0.0056	0.0050	25.0
		0.0054	24.0
		0.0068	23.0

Juvenile Survival

Two acute tests were run on juveniles: Acute 5 was run for 17 days in H_2S levels from 0.0014 to 0.0095 mg/liter and Acute 6 was run for 96 hr at levels from 0.0075 to 0.0292 mg/liter. In Acute 6 the LC50 was 0.0125 mg/liter H_2S at 96 hr. In Acute 5 the LC50 was 0.0130 mg/liter at 96 hr and 0.0086 mg/liter at 10 days. The LC50 did not change thereafter through 17 days of exposure (Table 92). At lower exposure levels in 17 days juveniles grew faster in weight than average of controls up to exposures of 0.0062 mg/liter. At 0.0095 mg/liter H_2S mean weight of fish was approximately half the mean of the controls.

CHRONIC TESTS

Experimental Design

Five chronic tests were run with rainbow trout; three with H_2S (Chronic 1, 3, and 4), one with phenol (Chronic 2), and one with combined H_2S and phenol (Chronic 5).

Chronic 1 was divided into three segments, a, b, and c. Diluter apparatus was the same as described for acute tests. In each segment of the tests four H_2S concentrations and one control were maintained (Table 93). Test chambers were 50 x 25 x 20 cm with 16-liter volume. Flow rates were 134 ml/min. Test 1-a was started with newly hatched fry, 1-b with 10-day-old fry, and 1-c with 50-day-old juveniles. The test was started with 30 fish per tank. They were fed Glencoe fry granules. Test chambers were illuminated with 40-watt incandescent lights on a 12-hr light cycle.

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Chronic 3 was started with eggs immediately after fertilization in the laboratory. One hundred and fifty eggs were placed in each of one control and four H_2S treatments (Table 94). For the first 33 days eggs and fry were held in cylinders 6 cm in diameter with Nitex screen bottoms. Fry were then transferred to 20-liter tanks described for

Table 92. LC50 VALUES FOR RAINBOW TROUT JUVENILES AT VARIOUS DAYS AND LENGTH AND WEIGHT AFTER 17 DAYS EXPOSURE TO DIFFERENT CONCENTRATIONS OF H_2S (ACUTE TEST 5)

H_2S concentration, mg/1	Mean length and weight (17 days), mm	g	LC50, mg/1 H_2S	(days)
Control	45.0	1.248	0.0130	(4)
Control	47.6	1.502	0.0113	(5)
Control	46.0	1.314	0.0104	(6)
0.0014	47.8	1.458	0.0103	(7)
0.0018	46.9	1.435	0.0102	(8)
0.0025	46.1	1.386	0.0091	(9)
0.0031	48.0	1.499	0.0086	(10)
0.0062	46.9	1.367	0.0088	(11)
0.0075	42.8	0.945	0.0087	(12)
0.0095	38.5	0.674	0.0087	(17)

Table 93. TEST CONDITIONS IN CHRONIC TEST 1 WITH RAINBOW TROUT[a]

	Chamber				
Item	1	2	3	4	5
Test 1-a (100 days duration)					
$\bar{x}$ H_2S conc. (mg/l)	Control	0.0018	0.0032	0.0075	0.0131
H_2S std. dev. (mg/l)	-	0.0020	0.0024	0.0037	0.0063
$\bar{x}$ pH	7.72	7.74	7.77	7.79	7.80
$\bar{x}$ temperature (C)	14.4	14.6	14.7	15.0	14.7
$\bar{x}$ dissolved O_2 (mg/l)	10.1	9.8	9.7	9.9	9.7
Test 1-b (90 days duration)					
$\bar{x}$ H_2S conc. (mg/l)	Control	0.0011	0.0033	0.0048	0.0106
H_2S std. dev. (mg/l)	-	0.0011	0.0046	0.0027	0.0098
$\bar{x}$ pH	7.72	7.73	7.76	7.78	7.79
$\bar{x}$ temperature (C)	14.7	14.8	14.8	15.0	14.8
$\bar{x}$ dissolved O_2 (mg/l)	9.6	9.8	9.7	9.7	9.6
Test 1-c (50 days duration)					
$\bar{x}$ H_2S conc. (mg/l)	Control	0.0011	0.0037	0.0059	0.0102
H_2S std. dev. (mg/l)	-	0.0008	0.0062	0.0033	0.0115
$\bar{x}$ pH	7.82	7.80	7.81	7.82	7.82
$\bar{x}$ temperature (C)	14.6	15.0	14.9	15.0	15.0
$\bar{x}$ dissolved O_2 (mg/l)	9.6	9.4	9.4	9.4	9.4

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[a] 30 fish per chamber.

Table 94. TEST CONDITIONS IN CHRONIC TESTS 2, 3, AND 4 WITH RAINBOW TROUT

Item	Chamber				
	1	2	3	4	5
Test 2[a]					
$\bar{x}$ phenol conc. (mg/1)	Control	1.50	3.40	7.70	26.20
Phenol std. dev. (mg/1)	-	0.16	0.17	0.25	1.77
$\bar{x}$ pH	7.78	7.79	7.81	7.79	7.79
$\bar{x}$ temperature (C)	12.4	12.5	12.5	12.5	12.5
$\bar{x}$ dissolved O_2 (mg/1)	10.5	10.5	10.5	10.5	10.5
$\bar{x}$ total alkalinity (mg/1)	220	220	220	220	220
Test 3[b]					
$\bar{x}$ H_2S conc. (mg/1)	Control	0.0009	0.0020	0.0025	0.0052
H_2S std. dev. (mg/1)	-	0.0006	0.0015	0.0024	0.0059
$\bar{x}$ pH	7.78	7.75	7.75	7.75	7.78
$\bar{x}$ temperature (C)	13.5	14.0	13.9	13.5	13.6
$\bar{x}$ dissolved O_2 (mg/1)	9.8	9.8	9.8	9.8	9.8
$\bar{x}$ total alkalinity (mg/1)	216	216	216	216	216
Test 4[c]					
$\bar{x}$ H_2S conc. (mg/1)	Control	0.0012	0.0040	0.0062	0.0123
H_2S std. dev. (mg/1)	-	0.0012	0.0019	0.0017	0.0042
$\bar{x}$ pH	7.74	7.72	7.71	7.71	7.72
$\bar{x}$ temperature (C)	13.3	13.7	13.4	13.2	13.4
$\bar{x}$ dissolved O_2 (mg/1)	9.6	9.6	9.6	9.6	9.6
$\bar{x}$ total alkalinity (mg/1)	218	218	218	218	218

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[a] 40 days duration; 50 fish per chamber.
[b] 145 days duration; 150 fish per chamber.
[c] 111 days duration; 150 fish per chamber.

acute tests where they were held to day 56. At that time the fingerlings were transferred to 208 x 55 x 52 cm fiber glass tanks filled to contain 503 liters of water. Fish were fed as in chronic test 1 and remained in these tanks to completion of test. Flow-through rate was 500 ml/min. A 12-hr light cycle was maintained with 200-watt incandescent lights.

Chronic 4 was started with 150 eyed eggs in one control and four H_2S treatments (Table 94). For the first 13 days eggs were held in cylinders as described for chronic 3. Fry were then transferred to 20-liter tanks as described for previous tests and fed as in previous tests and held to day 71 when fingerlings were placed in the fiber glass tanks previously described.

Chronic 2 was started with 50 eyed eggs placed in each of four concentrations of phenol and one control (Table 94). Eggs were held in cylinders as described above for 6 days and then transferred to 20-liter tanks where they were held to the end of the test (40 days). Flow rate was 500 ml/min. Tanks were illuminated as in chronic 3 and fish feeding was done as in chronic 1.

Chronic 5 was started with 50 juvenile fish in 20-liter tanks and exposed to H_2S for 74 days. One treatment with H_2S, one control, and four treatments with one concentration of H_2S combined with four concentrations of phenol constituted the test (Table 95).

Survival

Rainbow trout survival in 100 days when tests were started with newly hatched fry (1-a) was significantly affected at 0.0075 mg/liter but not at 0.0032 mg/liter H_2S (Table 96). At a concentration of 0.0132 mg/liter no fish survived to 28 days. When fish were started as 10-day-old fry (1-b), a marked reduction in survival was noted at 46 days in a concen-

Table 95. TEST CONDITIONS IN CHRONIC TEST 5 WITH RAINBOW TROUT[a]

Item	Chamber 1	2	3	4	5	6
$\bar{x}$ H_2S (mg/l)	Control	0.0052	0.0054	0.0056	0.0061	0.0048
$\bar{x}$ H_2S std. dev. (mg/l)	-	0.0018	0.0017	0.0016	0.0024	0.0020
$\bar{x}$ phenol (mg/l)	-	-	0.13	0.34	0.60	1.40
Phenol std. dev. (mg/l)	-	-	0.06	0.17	0.30	0.24
$\bar{x}$ pH	7.78	7.84	7.82	7.80	7.81	7.84
$\bar{x}$ temperature (C)	13.1	12.8	12.7	12.7	12.6	12.8
$\bar{x}$ dissolved O_2 (mg/l)	6.4	6.4	6.4	6.4	6.4	6.4
$\bar{x}$ total alkalinity (mg/l	223	223	223	223	223	223

[a] 74 days duration; 50 fish per chamber.

Table 96. SURVIVAL OF RAINBOW TROUT DURING CHRONIC EXPOSURE TO VARIOUS CONCENTRATIONS OF H_2S

(percentage)

Test	Stage at start	Days of exposure	H_2S (mg/l)				
			Control	0.0018	0.0032	0.0075	0.0132
1a	Newly hatched fry	28	100	100	100	97	0
		56	90	90	97	57	0
		84	90	87	97	57	0
		100	90	87	93	57	0
			Control	0.0011	0.0033	0.0048	0.0106
1b	10-day-old fry	18	100	100	97	100	83
		46	100	100	97	77	60
		74	97	97	87	74	33
		90	97	97	87	74	33
			Control	0.0011	0.0037	0.0059	0.0102
1c	50-day- old juveniles	6	100	97	100	93	97
		34	93	97	100	93	43
		50	93	97	100	90	43
			Control	0.0009	0.0020	0.0025	0.0052
3	Fertilized eggs[b]	28	56[a]	80	89	83	80
		56	34	75	71	80	15
		86	31	73	65	79	13
		114	31	73	62	78	9
		145	30	64	56	62	7
			Control	0.0010	0.0039	0.0063	0.0124
4	Eyed eggs[b]	27	58	49	60	56	55
		54	80	66	85	48	33
		82	52	42	54	46	4
		111	40	42	42	30	4

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[a] High mortality caused by excessive water agitation in chamber.

[b] Terminal stage - feeding larvae.

tration of 0.0048 mg/liter H_2S. In 90 days, survival was 33% at 0.0106 mg/liter and 87% at 0.0033 mg/liter H_2S. When fish were started as 50-day-old juveniles, survival was 43% in 50 days at 0.0102 mg/liter H_2S with no appreciable mortality at the lower levels.

In chronic 3 started with fertilized eggs, some reduction in survival after 28 days occurred at all concentrations from 0.0009 to 0.0052 mg/liter H_2S. After 56 days, survival was 15% of eggs at start with a concentration of 0.0052 mg/liter. Appreciable mortality occurred in control during egg stage and in H_2S treatments most mortality occurred during fry stage.

These data indicate that when rainbow trout are subjected to H_2S from fertilization onward any concentration above 0.0025 mg/liter (chronic 3) will cause some mortality. When fish are started as eyed eggs (chronic 4) they are more resistant and show no effect on survival at 0.0039 mg/liter but some effect at 0.0063 mg/liter H_2S.

Growth

Growth of fry was retarded at 0.0075 mg/liter H_2S and higher (chronic 1-a) (Tables 97 and 98). In the test started with fertilized eggs growth was retarded at all test levels. The apparent better growth at 0.0052 mg/liter H_2S was caused by survival of only a few large fish. When a test was started with eyed eggs (chronic 4), there appeared to be some increased growth at 0.0010 mg/liter H_2S but retardation at 0.0063 mg/liter and higher.

Tests with Combined Phenol and H_2S

As a preliminary to testing the chronic effects of combined H_2S and phenol, a 40-day test on eyed eggs was run on phenol (chronic 2) at four concentrations from 1.50 to 26.20 mg/liter (Table 99). At all concentrations tested survival and growth were affected. At 26.20 mg/liter no fry survived at 40 days.

Table 97. MEAN WEIGHT OF RAINBOW TROUT AFTER VARIOUS PERIODS OF EXPOSURE TO DIFFERENT CONCENTRATIONS OF H_2S

(grams)

Test	Stage at start	Days of exposure	H_2S, mg/l				
			Control	0.0018	0.0032	0.0075	0.0132
1a	Newly	57	0.408	0.491	0.491	0.327	-
	hatched	85	1.279	1.608	1.499	1.229	-
	fry	100	2.410	2.609	2.339	1.905	-
			Control	0.0011	0.0033	0.0048	0.0106
1b	10-day-old	47	0.576	0.627	0.555	0.506	0.562
	fry	75	1.304	1.477	1.524	1.442	1.550
		90	2.053	1.918	2.134	1.548	2.472
			Control	0.0011	0.0037	0.0059	0.0102
1c	50-day-old	6	0.435	0.403	0.441	0.354	0.382
	juveniles	34	1.286	1.160	1.203	1.505	1.252
		50	1.952	1.763	1.854	1.887	1.724
			Control	0.0009	0.0020	0.0025	0.0052
3	Fertilized eggs	145	7.900	6.180	6.290	5.960	8.680
			Control	0.0010	0.0039	0.0063	0.0124
4	Eyed eggs	111	4.920	5.110	4.320	2.460	1.670

Table 98. MEAN LENGTH OF RAINBOW TROUT JUVENILES AFTER VARIOUS PERIODS OF EXPOSURE TO DIFFERENT CONCENTRATIONS OF H_2S (millimeters)

Test	Stage at start	Exposure, days	H_2S, mg/l				
			Control	0.0018	0.0032	0.0075	0.0132
1a	Newly hatched fry	57	3.35	3.57	3.57	3.25	-[a]
		85	4.80	5.19	5.10	4.62	-
		100	5.69	5.87	5.65	5.65	-
			Control	0.0011	0.0033	0.0048	0.0106
1b	10-day-old fry	47	3.76	3.91	3.80	3.62	3.76
		75	4.86	5.18	5.20	5.00	5.26
		90	5.52	5.67	5.68	5.33	6.11
			Control	0.0011	0.0037	0.0059	0.0102
1c	50-day-old juveniles	6	3.46	3.47	3.45	3.25	3.35
		34	4.79	4.72	4.79	4.80	4.75
		50	5.35	5.34	5.38	5.36	5.37
			Control	0.0009	0.0020	0.0025	0.0052
3	Fertilized eggs	145[b]	8.90	8.10	8.30	8.00	9.30
			Control	0.0010	0.0039	0.0063	0.0124
4	Eyed eggs	111[b]	7.50	7.60	7.10	6.40	5.20

[a] All fry dead at 57 days.
[b] Terminal stage - juveniles.

Table 99. SURVIVAL, WEIGHT, AND LENGTH IN CHRONIC TEST 2 OF RAINBOW TROUT STARTED AS EYED EGGS AND EXPOSED TO VARIOUS CONCENTRATIONS OF PHENOL

Item	Exposure, days	Phenol, mg/l				
		Control	1.50	3.40	7.70	26.20
Survival (%)	At hatch	94	94	94	94	64
	40	90	66	32	20	0
Weight (g)[a]	40	0.338	0.152	0.099	0.092	-
Length (mm)	40	29.9	24.2	24.4	22.6	-

[a] Wet weight.

Chronic 5 with 0.0048-0.0061 mg/liter H_2S combined with four levels of phenol from 0.13 to 1.40 mg/liter (Table 100) showed no additive or synergistic effect on survival when fish were exposed to the two materials at the same time. Growth rate was slowed by H_2S alone at 0.0052 mg/liter and with 1.40 mg/liter phenol added a further retardation occurred. Although the two materials acting together at this concentration of phenol resulted in a greater retardation than either alone, the retardation was not as much as would have been expected from results of H_2S and phenol tested separately.

SUMMARY

Sperm motility and egg fertilization were not adversely affected up to concentrations of 0.0589 mg/liter H_2S. LTC of fry at 20 days was 0.0056 mg/liter. Mean LC50 of juveniles at 96 hr was 0.0130 mg/liter and at 17 days was 0.0087 mg/liter H_2S. When trout are subjected to H_2S from fertilization onward, any concentration above 0.0025 mg/liter H_2S will have adverse effects on growth and survival. When eggs or fish are started in H_2S at the eyed egg stage or later they are more tolerant. Phenol and H_2S in combination increase mortality but not as much as anticipated from separate tests.

Table 100. SURVIVAL, LENGTH, AND WEIGHT OF RAINBOW TROUT[a] IN A MIXTURE OF H_2S AND PHENOL AFTER VARIOUS PERIODS OF EXPOSURE IN CHRONIC TEST 5

Item	Exposure, days	Chamber 1	2	3	4	5	6
H_2S (mg/l)		Control	0.0052	0.0054	0.0056	0.0061	0.0048
Phenol (mg/l)		Control	Control	0.13	0.34	0.60	1.40
Survival (%)	28	98	100	100	100	100	98
	74	98	100	100	100	92	98
Weight (g)[b]	18	2.89	2.50	2.67	2.51	2.42	2.29
	74	5.66	4.92	5.07	5.08	4.17	4.35
Length (mm)	74	7.9	7.5	7.5	7.7	7.1	7.3

[a] Test started with 49-day-old juveniles.
[b] Wet weight.

SECTION XI

WHITE SUCKER

(*Catostomus commersoni* (Lacépède))

A series of four acute tests were conducted on sucker juveniles and a series of three chronic tests were started from sucker fry. Excessive mortality in the three successive chronic attempts necessitated their termination. The experimental design for the acute tests was the same as that described for walleye juveniles. The length of test fish varied between experiments from 32 - 121 mm (Table 101). Test temperature was 20-20.1 C, oxygen levels 5.9-6.2 mg/liter and pH 7.8-7.9. The 96-hr LC50 values varied from 0.0185 to 0.0290, with a mean of 0.0219 mg/liter H_2S. Tests on eggs and fry were conducted as part of another study prior to the start of this project (Smith and Oseid[20]). They are reported in the summary tables in the subsequent discussion section.

Table 101. TEST CONDITIONS AND LC50 VALUES OF H_2S IN ACUTE TESTS WITH WHITE SUCKER

Test	Source	Number fish per chamber	Mean length, mm	Mean test conditions			LC50, mg/1 H_2S			
				Temp., C	O_2, mg/1	pH	24 hr	48 hr	72 hr	96 hr
1	Reared from eggs	10	32	20.0	6.2	7.8	-	0.0185	0.0185	0.0185
2	Bait dealer	3	123	20.0	6.1	7.9	-	-	0.0247	0.0193
3	" "	3	124	20.1	5.9	7.9	0.0290	0.0230	0.0208	0.0208
4	" "	3	121	20.1	6.0	7.9	-	-	-	0.0290

SECTION XII

CRAYFISH

(Procambarus clarkii (Girard) and Cambarus diogenes Girard)

Acute tests were run on Procambarus clarkii (Girard) to determine 96-hr LC50 and LTC of H_2S. Chronic tests at low levels determined the influence of H_2S on survival, growth, and reproduction. During chronic tests the sensitivity of various life history stages was determined. Cambarus diogenes Girard was subjected to acute tests only to determine the differences between northern and southern species.

ACUTE TESTS

Collection, Treatment, Holding, and Acclimation

Procambarus clarkii was trapped and shipped from a commercial crayfish farm near Fairbanks, Louisiana (Table 102). They were shipped as adults, subadults, and berried females by air freight in styrofoam coolers with wetted and frozen potato sacks. Transportation time from farm to holding tanks in the laboratory was 12-18 hr. All crayfish were treated on arrival with 0.2% formalin to remove external oligochaetes before acclimation and long-term holding. Berried females provided material for egg, larval, and juvenile tests. Juveniles were reared to a weight of 1-3 g at 15-18 C and fed Glencoe pellets before testing. Crayfish were reared to 5-7 mm for use in one test. Adults were held in fiber glass tanks and transferred to acclimation tanks as needed. Acclimation of juveniles and adults (Tests 6-21) was done in a stock tank coated with aluminum asphaltum. Cinder blocks were placed in the tank for cover and all size groups of crayfish were fed Glencoe pellets and lettuce ad

Table 102. STAGE OF CRAYFISH AT COLLECTION AND AT START OF ACUTE TESTS

Test	Stage at start[a]	Stage at collection	Shipment or collection date
Procambarus clarkii trapped in Fairbanks, LA			
1,2,3	Egg	Egg	9-8-72
4,5	Larvae	Egg	9-8-72
6	Juvenile	Egg	9-29-71
7-11	Juvenile	Egg	9-8-72
12	Subadult	Subadult	9-29-71
13-15	Subadult	Subadult	12-7-71
16-19	Subadult	Subadult	3-9-72
20,21	Subadult	Subadult	5-31-72
22-25	Adult	Adult	3-11-74
Cambarus diogenes seined in Dakota County, MN[b]			
26	Juvenile & subadult	Juvenile & subadult	6-4-74
27,28	Juvenile & subadult	Juvenile & subadult	5-11-74

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[a] See glossary.
[b] A permanent pond.

libitum. All crayfish were acclimated for 8 to 16 days to test temperatures. Juveniles (tests 6-11) were held in 25-liter glass aquaria and fed Glencoe pellets twice per day. Eggs and larvae (tests 1-5) were derived from berried females held in fiber glass tanks. These tanks received the control water from egg test diluters. Eggs were acclimated 1 day at temperature and larvae were collected and used after eggs were hatched. *Cambarus diogenes* (tests 26-28) were collected from a Dakota County, Minnesota pond by hand seine and transported to the laboratory in an aerated stock tank. They were given 10 min treatment with 0.2% formalin solution and transferred immediately to stock tanks for acclimation. Acclimation time varied from 10 to 18 days at test temperatures and crayfish were fed Glencoe pellets and lettuce *ad libitum*.

Egg Tests

Eggs of *P. clarkii* were taken from berried females, acclimated for 1 day at test temperature and then tested at three temperatures (Table 103). Twenty-five eggs each were placed in 1-3/4-inch diameter acrylic cylinders with 1/16-inch mesh Nitex screen bottoms and outlets. The cylinders were attached to the outlets of the glass aquaria receiving the flow from diluters described for previous tests using sodium sulfide. The entire water volume from each cycle passed through the test chambers. A total of eight H_2S treatments and two controls were used for the egg tests. Ninety-five percent turnover time in the tanks at the head of each cylinder was 4 hr. H_2S concentration was determined from samples taken at the inlet to the cylinders. Tests were conducted for 25, 35, and 54 days at 14.2, 18.0, and 21.9 C, respectively. Photoperiod was 12 hr of light and 12 hr of darkness. Hatching was considered to have occurred with the breaking of the egg shell.

Twenty-six day LC50 values of H_2S were >0.408, >0.433, and 0.370 mg/liter H_2S at 14.2, 18.0, and 21.9 C, respectively. LTC's were 0.282, 0.208, and 0.131 mg/liter H_2S at the same test temperatures. Percentage hatch in 6-13 days at 21.9 C was 32-37% of controls at concentrations of H_2S

up to 0.266 mg/liter (Table 104). Corrected hatch at 18 C in 19 days was 89% with 0.188 mg/liter H_2S and in 26 days was 20% with 0.270 mg/liter. Corrected hatch was 76% at 0.208 mg/liter H_2S and 14.4 C in 29 days and 33% at 0.395 mg/liter H_2S and 14.0 C in 26 days.

Larval Tests

Two tests (acute tests 4 and 5) were run on first two instar larvae for 96 hr at 22 and 18 C. The same equipment and conditions were used as described for egg tests. Ninety-six-hr LC50 was 0.058 mg/liter H_2S at 22 C and 0.125 mg/liter at 17.9 C (Table 103).

Juvenile Tests

Juvenile test 6 was done on the H_2S gas apparatus previously described using one control and five treatments. Crayfish were acclimated for 2 days at 25 C and oxygen saturation. Mean test conditions were 24.6 C and 4.08 mg/liter O_2 with eight animals per chamber. Test chambers were 20-liter tanks with a 95% water replacement in 32 hr. Juvenile tests 7-11 were run in 20-liter tanks served by proportional diluters with sodium sulfide. Ninety-five percent replacement time was 2 hr. Ten crayfish were placed in each of one control and five treatments. Tests were conducted at temperatures of 22.1, 18.0, 14.1, and 13.9 C with acclimation for 10 days at 22, 18, and 14 C. Photoperiods were 12 hr of light and 12 hr of darkness. Cover was provided in the tanks by cement-asbestos tiles and fish were fed with Glencoe pellets after the first 96 hr. Ninety-six-hr LC50 at 22.1 C was 0.034 mg/liter H_2S, at 18 C was 0.083 mg/liter, and at 14.1 C was 0.147 mg/liter (Table 103). LTC at 18 C in 14 days was 0.053 mg/liter and at 13.9 C in 11 days was 0.126 mg/liter H_2S. Small crayfish (5-7 mm total length) in test 6 had 96-hr LC50 of 0.051 mg/liter H_2S at 24.6 C.

Subadult Tests

Ten acute tests were run (tests 12-21) in the same equipment as for

Table 103. ACUTE TEST CONDITIONS AND LC50 VALUES FOR CRAYFISH IN H_2S^a

Test	Stage	Days from collection to start	Number/ chamber	Mean carapace length, mm	Mean weight,^{b} g	Mean temperature, C	Mean pH	Mean O_2, mg/1	LC50, mg/1 H_2S 96 hr	LC50, mg/1 H_2S LTC (days)
				Procambarus clarkii						
1	Egg	3	25	-	-	21.9	7.67	5.74	0.370	0.151 (25)
2	Egg	3	25	-	-	18.0	7.68	6.40	>0.433	0.208 (35)
3	Egg	3	25	-	-	14.2	7.69	6.95	>0.408	0.282 (54)
4	Larvae	Reared from eggs	25	-	-	22.0	7.69	6.71	0.058	-
5	Larvae	"	25	-	-	17.9	7.62	7.20	0.125	-
6	Small juvenile	"	8	5-7	-	24.6	7.76	4.08	0.051	-
7	Juvenile	"	10	17.2	1.07	22.1	7.71	6.49	0.034	0.034 (4)
8	Juvenile	"	10	21.0	1.96	18.0	7.69	6.84	0.083	-
9	Juvenile	"	10	21.5	1.98	18.0	7.70	6.79	-	0.053 (14)
10	Juvenile	"	10	21.9	2.25	14.1	7.67	6.41	0.147	-
11	Juvenile	"	10	17.3	1.08	13.9	7.69	7.95	-	0.126 (11)
12	Sub-adult	12	6	39.4	15.3	25.9	7.82	4.15	0.075	-
13	Sub-adult	12	5	48.4	30.1	18.2	7.81	5.61	0.080	-
14	Sub-adult	12	5	49.9	35.0	18.0	7.83	5.48	0.095	-

Table 103 (continued). ACUTE TEST CONDITIONS AND LC50 VALUES FOR CRAYFISH IN H_2S^a

Test	Stage	Days from collection to start	Number per chamber	Mean carapace length, mm	Mean weight,^{b} g	Mean temperature, C	Mean pH	Mean O_2, mg/l	LC50, mg/l H_2S 96 hr	LC50, mg/l H_2S LTC (days)
15	Sub-adult	23	6	49.4	33.8	18.2	7.83	5.20	0.090	0.052 (10)
16	Sub-adult	23	8	44.7	35.5	18.0	7.82	5.42	0.100	-
17	Sub-adult	23	8	46.7	32.6	17.9	7.78	5.80	-	0.060 (11)
18	Sub-adult	27	8	40.9	36.8	17.9	7.76	5.06	0.115	-
19	Sub-adult	40	8	43.7	40.0	18.1	7.83	4.96	0.093	-
20	Sub-adult	19	7	41.4	37.6	17.9	7.81	6.51	0.091	-
21	Sub-adult	48	10	41.5	39.1	18.1	7.83	4.96	0.081	0.058 (15)
22	Adult	14	10	58.5	40.4	21.7	7.64	6.11	0.121	0.121 (4)
23	Adult	50	10	56.3	42.7	18.1	7.67	6.47	0.215	-
24	Adult	25	10	58.1	45.3	18.1	7.67	6.88	-	0.130 (11)
25	Adult	59	10	57.8	44.0	14.0	7.65	7.15	0.271	0.202 (10)
				Cambarus diogenes						
26	Sub-adult	9	10	26.4	5.2	22.0	7.70	5.95	0.070	-
27	Sub-adult	14	10	25.2	4.1	18.1	7.69	7.45	0.108	-
28	Sub-adult	18	10	24.7	4.4	13.9	7.68	7.60	0.150	-

[a] Acclimation ranged from 8 to 16 days; 1 day at temperature for eggs.
[b] Wet weight.

Test 104. HATCHING SUCCESS OF *Procambarus clarkii* EXPOSED TO H_2S AT VARIOUS TEMPERATURES

Test	H_2S, mg/l	Temperature, C	Number of eggs	Hatch,[a] %	Corrected hatch,[b] %	Time to 50% hatch, days
1	Control	21.9	25	76	100	8
	Control	21.8	25	72	100	9
	0.156	21.6	25	24	32	12
	0.176	21.9	25	28	37	15
	0.208	21.6	25	0	0	-
	0.221	21.8	25	0	0	-
	0.266	21.9	25	26	34	12
	0.324	22.0	25	0	0	-
	0.377	22.1	25	0	0	-
	0.389	21.9	25	0	0	-
2	Control	18.0	25	64	100	14
	Control	18.1	25	63	100	13
	0.170	18.1	25	55	87	20
	0.188	18.2	25	56	89	19
	0.214	18.0	25	0	0	-
	0.270	18.0	25	5	20	26
	0.279	18.1	25	0	0	-
	0.305	18.0	25	1	4	31
	0.402	17.9	25	0	0	-
	0.433	18.0	25	0	0	-
3	Control	14.0	25	87	100	25
	Control	14.3	25	83	100	23
	0.159	14.4	25	92	100	23
	0.208	14.4	25	63	76	29
	0.237	14.4	25	26	31	43
	0.246	14.0	25	68	78	25

Table 104 (continued). HATCHING SUCCESS OF Procambarus clarkii

EXPOSED TO H_2S AT VARIOUS TEMPERATURES

Test	H_2S, mg/l	Temperature, C	Number of eggs	Hatch,[a] %	Corrected hatch,[b] %	Time to 50% hatch, days
3	0.263	14.4	25	44	53	31
	0.336	14.0	25	52	60	44
	0.395	14.0	25	29	33	26
	0.408	14.0	25	0	0	-

[a] Missing eggs due to diluter malfunction were removed from consideration in calculation of % hatch.

[b] Hatch was corrected to 100% of controls.

juvenile test 6 except that 25-liter tanks with a 4-hr 95% water turnover time were used. One test (test 12) was run at 25.9 C and the others at 17.9 to 18.2 C. In the various tests five to ten individuals were placed in each of one control and five treatments. Acclimation at test temperature prior to testing was for 8-16 days. Glencoe pellets were fed after 96 hr of treatment. Photoperiod was 12 hr of light and 12 hr of darkness. Ninety-six-hr LC50 was 0.075 mg/liter H_2S at 25.9 C and at 17.9-18.2 C was 0.080-0.115 mg/liter, with a mean of 0.093 mg/liter H_2S (Table 103). LTC was 0.052-0.060 mg/liter H_2S in 10-15 days at 17.9-18.1 C.

Adult Tests

Four tests (tests 22-25) were run on adults at temperatures from 14.0 to 21.7 C. Ten individuals were placed in each of one control and six treatments in 70-liter stock tanks served by proportional diluters and sodium sulfide. Cement-asbestos tile shelters were provided in the tanks. Acclimation was at 14, 18, and 22 C for 10-16 days. Photoperiod was 12 hr of light and 12 hr of darkness. Crayfish were fed Glencoe pellets after 96 hr. Ninety-six-hr LC50 was 0.121 mg/liter H_2S at 21.7 C, 0.215 mg/liter at 18.1 C, and 0.271 mg/liter at 14.0 C (Table 103). LTC at 18.1 C was 0.130 mg/liter H_2S at 11 days and 0.202 mg/liter at 14.0 C in 10 days. LTC at 21.7 C was the same as 96-hr LC50.

Subadult Tests with *Cambarus diogenes*

Three tests were conducted on subadult *Cambarus diogenes* with ten individuals in each of one control and six test chambers. The 96-hr LC50 was determined at 13.9, 18.1, and 22.0 C (Table 103). Equipment was identical to that described for adult tests with *Procambarus*. The 96-hr LC50 at 13.9 C was 0.150 mg/liter H_2S, at 18.1 C was 0.108 mg/liter, and at 22.0 C was 0.070 mg/liter.

Summary of Acute Tests

In *Procambarus clarkii*, eggs are the most resistant life history stage with LTC vaues varying from 0.151 mg/liter H_2S at 21.9 C to 0.282 mg/liter at 14.2 C. The juvenile stage is the least resistant with LTC's varying from 0.034 mg/liter H_2S at 22.1 C to 0.126 mg/liter at 13.9 C. Yolk-sac larvae are intermediate in sensitivity between eggs and juveniles with 96-hr LC50 of 0.058 mg/liter H_2S at 22.0 C and 0.125 mg/liter at 17.9 C. Subadults have the same resistance as juveniles with LTC values varying from 0.052 to 0.060 mg/liter H_2S at 17.9-18.2 C. Adults were more resistant than all stages except eggs with LTC's of 0.121 mg/liter at 21.7 C and 0.202 mg/liter H_2S at 14 C. The *Cambarus diogenes* subadults tested at 18.1 C had similar sensitivity to *P. clarkii* subadults at 96 hr (0.108 and 0.093 mg/liter H_2S, respectively).

CHRONIC TESTS

Experimental Design

Three chronic tests were conducted on *Procambarus clarkii*. Chronic test 1 (1-a and 1-b) was run on two generations for 447 days. Test 1-a was run for the entire period and test 1-b was started after the 335th day in the same tanks using offspring of females of the original test. Temperature and daylight were carried in accordance with seasonal changes in Louisiana where the original stock was collected. The temperature varied from 9 to 24 C and light periods from 14 to 10.5 hr of light (Table 105). The intensity varied from 62 to 153 ft candles. The five test tanks were of fiber glass with a surface of 1.144 m^2 and a volume of 172 liters. Two berried females were placed in each tank. The number of young produced was 244, 148, 272, 54, and 161 for tanks 1-5, respectively. After 138 days the total number in each tank was reduced to 50 and at 168 days to 20 individuals. Cover was provided by cement-asbestos tiles and Glencoe pellets and lettuce were available continuously *ad libitum*. The four treatments ranged from 0.0041 to 0.0183 mg/liter H_2S for the first 364 days and from 0.0048 to 0.0158 mg/liter for the last 112 days. In 1-b, which ran for 112 days, the two treatments were 0.0048 and 0.0097 mg/liter H_2S. There was no reproduction in the two highest treatments.

Table 105. TEST CONDITIONS IN CHRONIC TESTS OF CRAYFISH (*Procambarus clarkii*)

Test conditions	Diluter 4	Diluter 5	Diluter 1	Diluter 2	Diluter 3
	Test 1-a (364 days)				
$\bar{x}$ H_2S conc. (mg/l)	Control	0.0041	0.0086	0.0135	0.0183
Std. Dev.	-	0.0018	0.0040	0.0060	0.0081
$\bar{x}$ temperature (C)	18.1	18.2	18.0	18.1	18.2
Range	9.1-24.3	10.0-24.1	9.8-23.9	9.8-24.1	9.8-24.1
$\bar{x}$ pH	7.71	7.70	7.69	7.69	7.73
Range	7.39-8.17	7.40-8.17	7.42-8.02	7.40-8.15	7.39-8.19
$\bar{x}$ dissolved O_2 (mg/l)	7.46	6.80	6.58	6.41	6.35
Range	5.7-9.9	4.2-9.7	3.7-9.6	3.5-9.6	2.7-9.5
$\bar{x}$ total alkalinity	201.2	201.2	201.2	201.2	201.2
Range	175-235	175-235	175-235	175-235	175-235
	Test 1-b (112 days)				
$\bar{x}$ H_2S conc. (mg/l)	Control	0.0048	0.0097	0.0127[a]	0.0158[a]
Std. Dev.	-	0.0023	0.0035	0.0062	0.0076
$\bar{x}$ temperature (C)	16.0	16.0	15.8	21.6	21.6
Range	8.8-24.3	9.0-24.1	8.8-23.9	20.0-24.1	20.1-24.1
$\bar{x}$ pH	7.71	7.69	7.69	7.69	7.76
Range	7.34-7.84	7.34-7.81	7.35-7.82	7.34-7.82	7.66-7.89
$\bar{x}$ dissolved O_2 (mg/l)	8.05	6.96	6.78	4.37	4.76
Range	6.3-10.9	4.7-10.2	4.6-9.9	3.6-4.6	2.7-6.0
$\bar{x}$ total alkalinity	203.2	203.2	203.2	203.2	203.2
Range	170-225	170-225	170-225	170-225	170-225

Table 105 (continued). TEST CONDITIONS IN CHRONIC TESTS OF CRAYFISH (*Procambarus clarkii*)

Test conditions	Diluter				
	1	4	2	3	5
	Test 2 (196 days)				
$\bar{x}$ H_2S conc. (mg/1)	Control	0.0044	0.0078	0.0140	0.0199
Std. Dev.	-	0.0033	0.0047	0.0082	0.0093
$\bar{x}$ temperature (C)	14.6	14.2	14.2	14.1	14.2
Range	9.8-23.9	9.1-24.2	9.8-24.0	9.8-24.0	10.0-23.8
$\bar{x}$ pH	7.74	7.72	7.73	7.72	7.76
Range	7.62-8.05	7.60-8.11	7.60-8.09	7.60-8.10	7.60-8.10
$\bar{x}$ dissolved O_2 (mg/1)	8.40	6.44	7.02	6.78	7.63
Range	4.7-9.6	5.8-9.9	4.6-9.6	3.8-9.5	4.4-9.7
$\bar{x}$ total alkalinity	216.5	216.5	216.5	216.5	216.5
Range	200-235	200-235	200-235	200-235	200-235
	Test 3[a] (196 days)				
$\bar{x}$ H_2S conc. (mg/1)	Control	0.0044	0.0088	0.0115	0.0172
Std. Dev.	-	0.0040	0.0069	0.0087	0.0146
$\bar{x}$ temperature (C)	20.76	20.63	20.67	20.61	20.59
Range	19.3-25.7	19.2-25.9	19.2-25.8	19.1-25.0	18.8-25.1
$\bar{x}$ pH	7.73	7.75	7.73	7.73	7.73
Range	7.50-7.98	7.58-8.09	7.54-8.05	7.58-8.08	7.56-8.00
$\bar{x}$ dissolved O_2 (mg/1)	6.86	6.02	5.97	5.36	6.31
Range	4.00-8.65	5.15-7.70	4.10-8.05	3.00-7.95	4.10-7.90
$\bar{x}$ total alkalinity	210.6	210.6	210.6	210.6	210.6
Range	198-231	198-231	198-231	198-231	198-231

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[a] Data from first 28 days only.

Chronic test 2 ran for 196 days under the same seasonal conditions as described for test 1. Light intensity was 34-40 ft candles and test tanks were 40-gal stock tanks containing 140 liters of water. The test was started with 20 juveniles 5-10 mm total length in each tank. Cover was provided by cement-asbestos tiles and Glencoe pellets and lettuce were fed ad libitum. H_2S treatments ranged from 0.0044 to 0.0199 mg/liter.

Chronic test 3 was conducted for 196 days at a temperature of 25 C for the first 34 days and at 20 C for the remainder of the run. Photoperiod was 12 hr of light and 12 hr of darkness. The light intensity was 34-37 ft candles. H_2S treatments ranged from 0.0044 to 0.0172 mg/liter.

Survival

Survival in chronic test 1-a which was started with larvae from berried females varied from 28% for 447 days in the control to 1% after 332 days at 0.0183 mg/liter H_2S (Table 106). Percentages were derived from the product at the end of each culling period. At 232 days there was reduction at the three highest levels. The first marked loss was at the juvenile stage after 232 days in all treatments and control. The second generation (test 1-b) survival was 40% in the controls and 67.6% at 0.0097 mg/liter H_2S. In chronic test 2 after 196 days survival was 100% at 0.0078 mg/liter H_2S and 54% at 0.0199 mg/liter. In chronic test 3 after 196 days survival in the control was 86% and at 0.0172 mg/liter H_2S was 11%. The no-effect level for survival in test 1-a after 447 days was 0.0041 mg/liter H_2S and in test 1-b after 112 days was 0.0097 mg/liter since higher levels were not tested. In test 2 the no-effect level was 0.0078 mg/liter and in test 3, 0.0088 mg/liter H_2S at 196 days.

Reproduction

Reproduction in chronic test 1-a was derived from 1 to 3 surviving berried females at 235 days (Table 107). One female surviving at a concentration of 0.0183 mg/liter H_2S had no eggs attached. Of three females

Table 106. SURVIVAL OF CRAYFISH (*Procambarus clarkii*) WITH LONG-TERM EXPOSURE TO H_2S (expressed as percentage[a])

Test	Exposure, days	H_2S, mg/liter				
		Control	0.0041	0.0086	0.0135	0.0183
1-a (first generation)[b]	55	98	92	96	100	26
	139	94	89	91	82	13
	232	70	71	46	53	3
	322	37	36	27	16	1
	447	28	27	14	12	1
		Control	0.0048	0.0097	0.0127	0.0158
1-b (second generation)	112	40	42.3	67.6	-	-
		Control	0.0044	0.0078	0.0140	0.0199
2	28	94	100	100	95	94
	84	77	100	100	79	73
	140	73	100	100	69	69
	196	70	100	100	64	54
		Control	0.0044	0.0088	0.0115	0.0172
3	28	100	100	100	100	100
	84	91	73	100	63	50
	140	89	60	90	31	11
	196	86	60	83	31	11

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[a] Corrected for loss from culling and sampling.
[b] First generation derived from hatched larvae.

Table 107. REPRODUCTION OF CRAYFISH (*Procambarus clarkii*) IN CHRONIC TEST 1

Item	Diluter				
	4	5	1	2	3
H_2S conc. (mg/liter)	Control	0.0041	0.0086	0.0135	0.0183
Number berried females	2	2	2	2	2
Number of larvae[a]	54	161	249	148	272
Survival - 447 days (%)	28	27	14	12	1
Total number of females alive at end of test	2	4	3	3	1
Total number berried females	2	3	2	1[b]	0
Total weight berried females (g)	38.7	48.3	30.2	27.8	0
Total number eggs	120	201	102	35	0
Number eggs/berried female	60	67	51	35	0
Number eggs/g berried female	3.10	4.17	3.38	1.26	0
H_2S conc. (mg/liter) - second generation	Control	0.0048	0.0097	0.0127	0.0158
Survival (%) - second generation (112 days)	40.0	42.3	67[illegible]	0	-

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[a] Numbers culled to 50 at day 138 and to 20 at day 168; percentage adjusted to numbers before culling.

[b] Eggs produced were infertile.

at 0.0133 mg/liter H_2S, only one produced eggs and they were infertile. At 0.0086 mg/liter, two of the three surviving females and at 0.0041 mg/liter, three of four surviving females were berried. The two surviving females in the control were berried. The number of eggs per gram of berried female varied from 3.10 in the controls to 1.36 at 0.0133 mg/liter H_2S. As noted above, the eggs at this latter concentration were infertile. Survival of eggs and survival of larvae from eggs through 112 days in the second generation were 40.3 to 67.6%. No detriment to survival was noted in the three test levels examined. The safe concentration of H_2S for reproduction appears from the foregoing data to be 0.0086 mg/liter. At higher concentrations eggs are either infertile or none are produced.

Growth

In chronic test 2 growth was inhibited at 0.0078 mg/liter H_2S after 196 days (Table 108). In chronic test 3 growth was inhibited after 196 days at 0.0088 mg/liter. The no-effect concentration of H_2S on growth was 0.0044 mg/liter in chronic tests 2 and 3.

Acute 96-hr tests were run on subsamples of chronic 1-a and 1-b organisms (Table 109). All tests were conducted at 18 C. The 96-hr LC50 for second generation controls is 0.0800 mg/liter H_2S. In the first generation LC50 values were higher in individuals taken from the higher concentration, indicating some degree of acclimation. At concentrations of 0.0041 mg/liter H_2S, the LC50 was approximately the same as found in the more extensive acute tests reported above. In the second generation (1-b) LC50's were lower at comparable concentrations than in the first generation (1-a).

Summary of Chronic Tests

The no-effect concentration among the levels tested was 0.0041 mg/liter H_2S and was based on both survival or gain in mean weight in tests 1a and 2. Among survivors and without reference to growth rate, the no-effect level for reproduction was 0.0086 mg/liter H_2S.

Table [illegible]. WEIGHT OF CRAYFISH ([illegible]) WITH LONG-TERM EXPOSURE TO H_2S

(grams)

Test	Exposure, days	H_2S, mg/liter				
		Control	0.0041	0.0086	0.0135	0.0183
1-a	125	0.47	0.25	0.12	0.18	0.24
First generation	139	0.69	0.36	0.18	0.26	0.32
	169	1.99	1.30	0.70	0.80	1.07
	204	6.91	4.49	2.99	3.38	2.37
	232	11.26	6.91	5.47	5.92	7.33
	447	28.74	18.11	20.89	27.65	14.63
1-b		Control	0.0048	0.0097	0.0127	0.0158
Second generation	112	0.50	0.47	0.11	-	-
2		Control	0.0044	0.0078	0.0140	0.0199
	28	0.14	0.26	0.21	0.11	0.11
	84	0.39	0.31	0.43	0.27	0.24
	140	1.56	1.59	1.37	0.89	0.61
	196	11.22	10.76	7.57	7.57	5.22
3		Control	0.0044	0.0088	0.0113	0.0171
	28	0.39	0.34	0.32	0.25	0.22
	84	3.63	4.39	3.53	2.58	2.38
	140	12.53	14.09	7.20	12.30	12.84
	196	16.19	17.43	11.49	15.11	14.93

Table 109. 96-HOUR LC50 VALUES OF SUBSAMPLES OF JUVENILE CRAYFISH (*Procambarus clarkii*) AFTER LONG-TERM EXPOSURE TO H_2S

Test	Chronic exposure, mg/liter H_2S	96-hour LC50, mg/liter H_2S
1-a First generation	Control	-
	0.0041	0.1080
	0.0086	0.1140
	0.0133	0.1270
	0.0183	-
1-b Second generation	Control	0.0800
	0.0048	0.0950
	0.0097	0.1000

SECTION XIII

BENTHIC INVERTEBRATES

Laboratory studies on one isopod, *Asellus militaris* Hay, two amphipods, *Crangonyx richmondensis laurentianus* Bousfield and *Gammarus pseudolimnaeus* Bousfield, three Ephemeroptera, *Baetis vagans* McDonough, *Ephemera simulans* Walker, and *Hexagenia limbata* (Serville), were conducted. Field studies and surveys have indicated that H_2S is often present in both polluted and some natural ecosystems at levels that are detrimental to fish and invertebrates. Limited references in the literature to the effect of H_2S on various invertebrates suggested that a rigorous evaluation of acute toxicities in a representative series of aquatic organisms associated with fish populations would permit an overall evaluation of the importance of H_2S in the aquatic systems. The species used in these tests were selected to include organisms from a wide range of habitat conditions varying from clear, cool water and firm substrate to warm, turbid waters with substrates where insects might burrow in the mud and where H_2S levels might normally be relatively high. In addition to the two crayfish discussed in Section XII, a series of acute tests was run on six other species and chronic exposure tests were run on *Gammarus* and *Hexagenia*.

ACUTE TOXICITY TESTS

Experimental Design

The isopods, *Asellus militaris*, used in the experiments were collected from Jackfish Bay, Rainy Lake, Minnesota and had a mean length of 8 mm (range 5-13 mm). *Crangonyx* was also taken from Jackfish Bay and ranged from 6-15 mm in length, with a mean of 10 mm. *Gammarus* was collected in Valley Creek, Washington County, Minnesota and varied in size from 8-16 mm, with a mean of 11 mm. *Baetis* was also taken from Valley Creek and ranged in size from 4-6 mm, with a mean of 5 mm. Nymphs of *Hexagenia* varied from 14-35 mm, with a mean of 23 mm, and were taken from Jackfish Bay and Crystal Beach, Rainy Lake, Minnesota. *Ephemera* ranged from 13-21 mm, with a mean of 17 mm, and were taken from Crystal Beach. The organisms were collected with a Peterson dredge, hardware cloth scoop, or drift net. Organisms were held in the laboratory at the same temperature as tests and were fed detritus from the collection site.

All tests were conducted in flow-through apparatus as described by Colby and Smith[1] and Adelman and Smith[2]. The former apparatus consisted of a cylindrical chamber 3.8 cm in diameter by 9.5 cm deep with a Nitex screen at the bottom. The second type was an acrylic box 7.6 x 7.6 x 5 cm with various substrates into which the organisms could burrow. H_2S levels were maintained by dissolving H_2S gas in oxygen-free water and mixing this water at the entrance to the test chambers with a proportioned amount of oxygen-saturated water to achieve the desired oxygen and H_2S concentrations. After mixing, the solution passed through the test chamber in not more than 90 sec. Analyses of the H_2S content were routinely made at least once each day.

Tests set up to determine median tolerance limits consisted of five H_2S concentrations and one control (Table 110). Temperature and oxygen were adjusted to meet the requirements of the various tests as subsequently outlined. In some cases substrate, oxygen, and pH were varied while a constant level of H_2S was maintained. Experiments on the effect of feeding and non-feeding of *Gammarus* during bioassay and on the effect of H_2S on feeding characteristics were conducted.

Table 11D. SUMMARY OF ACUTE BIOASSAYS CONDUCTED WITH H_2S ON SIX SPECIES OF INVERTEBRATES

Species	Number of tests	Duration, days	Temperature, C	Range H_2S concentration, mg/l	LC50,[a] mg/l H_2S
Asellus	4	4	15.1	0.044–2.196	1.070–1.700
Crangonyx	8	4	15.1	0.029–2.671	0.310–0.840
Gammarus	10	4–10	12.4	0.008–0.112	0.030–0.059
Baetis	2	2–4	14.8	0.008–0.064	0.020–0.026
Ephemera	5	4–11	15.0	0.106–0.617	0.135–0.380
Hexagenia	39	2–11	15.0	0.005–0.702	0.026–0.680

[a] LC50 at conclusion of test period. Ranges represent all tests and all exposure times.

[illegible]

First tests with Hexagenia were done in cylinders (Colby and Smith[1]). Consideration of work by Eriksen[21,22] suggested that chamber design and substrate would have a significant effect on the sensitivity of an organism to a toxicant. A box was designed, therefore, which permitted inclusion of a substrate into which the insects could burrow (Adelman and Smith[2]). Eriksen[21] showed that oxygen consumption was 0.65 cc/g dry body weight/hr when nymphs were in burrows and 1.1 cc when on bare substrate. During the present study comparative tests with cylindrical chambers and boxes with substrate were made. With an oxygen level of 2 mg/liter and temperature of 15 C, the 48-hr LC50 for Hexagenia in cylinders was 0.460 mg/liter H_2S and in the box with substrate was 0.520 mg/liter H_2S. Oxygen at 2.0 mg/liter was selected for species collected in Rainy Lake because significant H_2S concentrations were usually not found where oxygen was higher (Colby and Smith[1]). The numbers of days to 50% and 25% survival in three pairs of box and cylinder tests under the same conditions were more than twice as great in boxes with mud substrate (Table 111). After 2 days of exposure, controls from nine tests were transferred to Nitex baskets with and without mud substrates for 6 days. When both experimental period and post-treatment periods were with mud substrate, the survival was greater (Table 112). In another test, mud substrate was provided in two series of H_2S concentrations with box chambers. In one set burrowing was prevented by an overlay of Nitex screen. Where burrowing was permitted, the 96-hr LC50 was 0.120 mg/liter H_2S and where prevented, 0.060 mg/liter H_2S. A 12-day test without H_2S was conducted in boxes with mud, with mud and screen to prevent burrowing, and with no mud. Where burrowing was permitted, survival was 62-75%. When burrowing was not permitted with mud present, survival was 0-12%. In tests without mud present, survival was 12-38%.

Type of Substrate--To determine whether type of mud substrate was a factor in resistance, two tests were run with sludge from below a paper mill and mud from an unpolluted habitat of Hexagenia. The test chamber

Table 111. SURVIVAL TIME OF *Hexagenia* IN DIFFERENT TYPE CHAMBERS WITH H_2S IN THREE PAIRED TESTS[a]
(days)

	Chamber					
	1		2		3	
Item	Cylinder	Box[b]	Cylinder	Box	Cylinder	Box
H_2S (mg/liter)	0.143	0.178	0.266	0.276	0.355	0.365
Survival time						
50% or more	3.5	8	3	6	2.5	4.5
25% or more	4.5	9.5	3.5	8.5	3.5	5.5

[a] 2 mg/liter O_2; 15 C.
[b] Box contained mud for burrowing.

Table 112. EFFECT OF CHAMBER TYPE ON *Hexagenia* SURVIVAL WITHOUT TOXICANT PRESENT[a]

Experimental chamber (2 days)[b]	Number of tests	Post-treatment Nitex basket chamber (6 days)[b]	Survival	
			Mean, %	Range, %
Cylinder without mud	2	Without mud	22.5	0-50
Box with mud[c]	2	Without mud	40.0	30-50
Box with mud	5	With mud	80.0	60-100

[a] All tests at 2.0 mg/liter O_2 and 15 C except as noted.
[b] 2 days of treatment, 6 days in post-treatment.
[c] One test at 4.0 mg/liter O_2.

was a trough, half of which contained sludge and half mud so that water flowed over both and nymphs had access to both. In the first test, after 3 days 78% of nymphs had selected the mud and in the second, after 4 days 87% selected the mud in preference to the sludge. In a subsequent 96-hr LC50 bioassay conducted over mud and sludge there was little difference with 0.320 mg/liter H_2S on mud and 0.310 mg/liter H_2S on sludge. Apparently the type of material into which burrows were made did not alter the reaction to H_2S. When *Crangonyx*, a species found where natural H_2S may be abundant, was tested in boxes with mud and in cylinders without mud, resistance to H_2S was greater when animals could burrow (Table 113).

Gammarus were tested to determine the effect of various substrates on resistance to H_2S. Box chambers with mud, fine sand, pebbles (diameter 1 cm), and no substrate except the box floor were used in a 96-hr test at 0.049 and 0.052 mg/liter H_2S with one control. Oxygen was 3.84 mg/liter and temperature 15.1 C. Percentage survival varied from 4% on mud to 36% on pebbles (Table 114). The course substrate had the highest survival rate and the finest (mud), the lowest. The observed reaction may have been related to reduced activity where suitable cover was available.

Area and Volume--In one test with *Gammarus* at 5.92 mg/liter O_2 and 14.8 C, designed to determine the effect of size of chamber on 96-hr LC50, an increase of tenfold in bottom area and threefold in volume decreased the 96-hr LC50 of H_2S approximately 25%. Other tests conducted by Siesennop[23] and Smith (unpublished data) where chamber sizes were 6 and 20 liters, respectively, the LC50 was depressed still further (Table 115). It is believed that increased area decreased activity and hence the LC50.

Effect of Water Quality

Oxygen--To determine the influence of ambient oxygen concentration on resistance to H_2S, *Gammarus*, *Ephemera*, and *Hexagenia* were subjected to

Table 113. LC50 VALUES OF H_2S TO *Gammarus* IN CYLINDERS AND BOX AND MUD CHAMBERS[a]

(mg/liter H_2S)

	LC50		
Chamber	48 hr	72 hr	96 hr
Cylinder	0.540	0.425	0.310
Box	0.770	0.590	0.510

[a] 2 mg/liter O_2, 15 C, pH 7.4; cylinders without mud; boxes with mud substrate.

Table 114. EFFECT OF SUBSTRATE TYPE ON SURVIVAL OF *Gammarus* AT SIMILAR H_2S LEVELS AND VARIED TIMES

(percentage survival)

	Survival				
H_2S conc. (mg/l)	Control	0.049	0.049	0.051	0.052
Substrate	None	Mud	Fine sand	None	Pebble[a]
24 hr	100	96	96	96	100
48 hr	100	20	24	48	100
72 hr	96	4	16	35	100
96 hr	96	4	8	26	36

[a] Diameter 1 cm.

Table 115. EFFECT OF CHAMBER AREA AND VOLUME ON 96-HR LC50 OF H_2S WITH *Gammarus*

Number of individuals	Bottom area, cm^2		Volume, cc		96-hr LC50,[a] mg/liter H_2S
	Total	Per ind.	Total	Per ind.	
25	11	0.44	107	4.3	0.059
25	116	4.6	325	13.0	0.044
20	400	20.0	6,600	330	0.035[b]
40	1,288	32.2	20,000	500	0.022[c]

[a] Oxygen and temperature were approximately 6 mg/liter and 15.0 C for all tests.

[b] Siesennop.[23]

[c] L. L. Smith, Jr. (unpublished data).

various levels of oxygen and H_2S. In the first series of tests *Gammarus* and *Ephemera* were treated with constant levels of H_2S and varied concentrations of oxygen. *Gammarus* was subjected to 0.094 mg/liter H_2S and 5.77, 4.86, 3.18, and 2.30 mg/liter O_2 at 11 C for 10 days or until all organisms were dead. At the highest oxygen concentration 80% survived for 10 days and at the lowest, 4% survived through 6 days. *Ephemera* were exposed to 0.20 mg/liter H_2S and 7.9, 6.1, 3.9, and 1.7 mg/liter O_2 at 15 C. With 7.9 mg/liter O_2 no mortality occurred in 10 days but at 1.7 mg/liter O_2 all died in 3 days. At intermediate oxygen levels 40% or more survived 10 days. *Hexagenia* were exposed to 2 and 4 mg/liter O_2 and five levels of H_2S from 0.076 to 0.880 mg/liter at 15 C in cylindrical chambers without mud (two replications). At 2 mg/liter O_2, LC50 was 0.620 and 0.460 mg/liter H_2S at 24 and 48 hr, respectively. In 4 mg/liter O_2, LC50 was 0.640 and 0.535 mg/liter H_2S at 24 and 48 hr.

A series of four tests on *Gammarus* at 6 and 4 mg/liter O_2 and at 10 and 15 C was run to determine the combined effect of the two variables on LC50 of H_2S. The tests were run for 10 days at five H_2S levels (0.011-0.075 mg/liter) in the cylindrical chambers without mud. After 10 days the LC50 varied only 0.007 mg/liter (Table 116). The lowest was 0.042 mg/liter H_2S at 4 mg/liter O_2 and 15 C.

At 10 C and 6 mg/liter O_2 early resistance was high (0.095 mg/liter H_2S) but decreased rapidly up to 6 days and little thereafter. At 15 C early resistance was less and after 6 days decreased slowly. With 4 mg/liter O_2 resistance to H_2S was less at the higher temperature. These data indicate that after initial difference in resistance, oxygen and temperature within the ranges tested do not have much influence on long-term response.

Effect of pH--The effect of pH on H_2S toxicity aside from its relationship to dissociation was considered to be substantial by Bonn and Follis[24] on the basis of 3-hr tests with fish. During the present study

Table 116. LC50 VALUES OF H_2S TO GAMMARUS AFTER VARIOUS INTERVALS AT 4 AND 6 MG/LITER O_2 AND 10 AND 15 C (mg/liter H_2S)

	6 mg/l O_2		4 mg/l O_2	
Days	10 C	15 C	10 C	15 C
2	0.095	0.071	-	0.062
4	0.059	0.059	0.054	0.058
6	0.053	0.056	0.051	0.052
8	0.050	0.054	0.050	0.045
10	0.049	0.045	0.049	0.042

with *Hexagenia* which were acclimated at test conditions, three 96-hr tests at 7.4 pH and five 96-hr tests at 7.7 pH run in winter had a mean LC50 of 0.365 and 0.151 mg/liter H_2S, respectively. Seven summer tests run at 7.7 pH and the same temperature had a mean 96-hr LC50 of 0.111 mg/liter H_2S. Oxygen concentration and temperature during all tests were 2.0 mg/liter O_2 and 15 C.

Effect of Laboratory Acclimation

A series of experiments were run to determine the effect of holding test organisms in the laboratory prior to bioassay tests. *Asellus*, *Ephemera*, and *Hexagenia* were held for varied lengths of time prior to the start of each test. Results indicated a marked influence of laboratory acclimation time on resistance when pH, temperature, and food availability were the same in comparative tests. *Asellus* were held in fresh water in the laboratory for 9, 16, 30, and 44 days prior to testing and then subjected to 96-hr LC50 tests. The LC50's were 1.07, 1.21, 1.52, and 1.70 mg/liter H_2S for succeeding acclimation periods. Temperature was 15 C, O_2 was 2 mg/liter and pH, 7.3-7.5. *Ephemera* were held for 2 and 17 days in fresh water at 15 C and 2 mg/liter O_2 before testing. After 5 days LC50's were 0.21 and 0.20 mg/liter H_2S. At 7 days LC50's were 0.20 and 0.14 mg/liter H_2S, respectively. *Hexagenia* were held in fresh water on mud substrate with plant and animal detritus for 3, 6, 10, 14, and 18 days and then transferred to Nitex baskets without food to determine subsequent survival rates in the absence of toxicants. Holding up to 6 days resulted in reduced survival after transfer to baskets but longer holding periods did not significantly increase mortality beyond initial losses (Table 117). These results suggest the advisability of a 6-day acclimation period prior to toxicity tests because reactions do not vary with longer holding time.

Effect of H_2S Acclimation

Because bottom-living organisms, which burrow in the substrate, may be exposed continuously to low levels of H_2S, two tests were run on *Hexa-*

Table 117. SURVIVAL TIME OF *Hexagenia* HELD ON MUD IN FRESH WATER FOR VARYING PERIODS AND THEN TRANSFERRED TO NITEX BASKETS WITHOUT FOOD OR TOXICANT

(days)

Holding period (days)	Survival rate in baskets		
	50%	10%	0%
3	10.2	10.8	11
6	5.5	8.5	9
10	7.2	7.8	8
14	4.5	7.5	10
18	5.6	11.5	13

genia to determine the effect of H_2S acclimation on acute response to H_2S. Test organisms were exposed in duplicate to 0.016 mg/liter H_2S, 2 mg/liter O_2, and 15 C at 7.7 pH for 13 days. The 96-hr LC50 was 0.108 mg/liter and 0.140 mg/liter H_2S. The 96-hr LC50 of organisms held in freshwater for the same period and tested simultaneously were 0.103 and 0.098 mg/liter H_2S. After 10 days of exposure to 0.036 mg/liter H_2S under the same conditions as the 13-day acclimation, the 96-hr LC50 was 0.108 mg/liter H_2S. Simultaneous tests with the organisms held in fresh water was 0.135 mg/liter H_2S. The data suggest that pretreatment with very low levels may increase resistance to acute levels of H_2S but that higher pretreatment levels increase sensitivity. The results are inconclusive but indicate the need for more careful evaluation of effects of low level exposure of bottom-living invertebrates on subsequent acute lethal tests.

Effect of Season, Sex and Size

It is usually more desirable to catch wild invertebrates shortly before testing rather than to maintain cultures. Because seasonal differences in resistance may occur, acute tests were run at different seasons from 1967-1969 on *Hexagenia* (Table 118). It is evident from the data that organisms taken in summer are more sensitive than those taken in fall and winter and also that wild organisms taken in different years may vary in sensitivity.

In seven H_2S bioassays with *Gammarus* ranging in size from 8.0 to 16.0 mm mortality rates of males and females were compared and size of mortalities and survivors noted. No significant difference in sensitivity to H_2S of males and females or different sizes was apparent. There was considerable variability between tests but no trends.

Effect of H_2S on Behavior

Behavioral effects of H_2S on *Ephemera* and *Hexagenia* were noted by observation of emergence of nymphs from burrows during 10-day tests in

Table 118. LC50 VALUES OF H_2S FOR *Hexagenia* COLLECTED IN DIFFERENT MONTHS[a]

(mg/liter H_2S)

Month	LC50 48 hr	LC50 72 hr	LC50 96 hr
1967-68			
August	0.23	0.16	0.12
October	0.40	0.18	0.13
December	0.46	0.26	0.16
February	0.39	0.24	0.10
July	0.25	0.17	0.12
1968-69			
November	0.16	0.16	-
January	-	0.14	0.09
March	-	0.17	0.12
May	-	0.11	0.07
July	0.13	0.06	0.03

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[a] Tests at 15 C, 2 mg/liter O_2, and 7.7 pH; acclimation 10 days at test conditions.

chambers with mud substrate at different concentrations of H_2S and O_2. Percentage of emergence was based on the total number of individuals in the test period. At an exposure of 0.20 mg/liter H_2S and four levels of O_2 from 7.9 to 1.7 mg/liter, emergence of Ephemera varied from 100% in 3 days at 1.7 mg/liter O_2 to no emergence in 9 days at 7.9 mg/liter O_2 (Table 119). At 2.00 mg/liter O_2 and five levels of H_2S from 0.16 to 0.30 mg/liter, emergence was earlier at the higher H_2S concentrations. With Hexagenia subjected to 2.0 mg/liter O_2 and five H_2S concentrations from 0.18 to 0.54 mg/liter, emergence of nymphs was sooner at higher concentrations (Table 120). In all test series using mud substrates only two nymphs died in burrows rather than emerging before death. The difference in days between 50% emergence of nymphs and 50% mortality of Ephemera varied from less than 1 day at 0.5 mg/liter H_2S to 4 days at 0.15 mg/liter. With Hexagenia the difference was 3 days throughout the same range of sulfide concentrations.

Effect of H_2S on Feeding of Gammarus

When Gammarus was fed Populus alba pyramidalis leaves and treated with 0.010-0.050 mg/liter H_2S at 10 C and 5.8-6.0 mg/liter O_2, food consumption varied from 0.431 mg/individual/day in controls to 0.135 mg/individual/day at 0.050 mg/liter H_2S (Table 121).

Summary of Acute Tests

From the foregoing data it is apparent that conditions under which a bioassay is run and the time of year when specimens are collected alter the response to H_2S. It is also important that an unnatural degree of activity in test organisms not be induced by test conditions. To determine a useful LC50 for purposes of habitat evaluation, the data from tests done under conditions in our judgment most similar to the natural habitat were selected (Table 122).

A comparison of relative sensitivity of the invertebrates tested showed Asellus to be the most resistant, followed by Crangonyx and the burrowing

Table 119. EMERGENCE OF *Ephemera*[a] WITH CONSTANT H_2S
AND VARIED OXYGEN CONCENTRATION AND WITH CONSTANT OXYGEN
AND VARIED H_2S CONCENTRATION IN SUCCEEDING DAYS
(expressed as percentage)

	O_2, mg/1[b]				H_2S, mg/1[c]				
Days	1.7	3.9	6.1	7.9	0.0	0.16	0.19	0.26	0.30
1	0	10	10	0	10	20	40	80	100
2	100	10	10	0	0	20	70	100	100
3	100	10	10	0	0	70	100	100	100
4	-[d]	10	20	0	10	30	100	100	-[d]
5	-	20	20	0	10	50	80	100	-
6	-	20	20	0	10	50	100	100	-
7	-	40	20	0	10	100	100	100	-
8	-	50	20	0	10	100	100	100	-
9	-	60	20	0	10	80	100	100	-
10	-	60	30	0	10	100	100	100	-

[a] Emergence of nymphs from burrows in two tests.
[b] With 0.20 mg/liter H_2S.
[c] With 2.0 mg/liter O_2.
[d] Dash indicates no data - all nymphs emerged and died.

Table 120. EMERGENCE[a] OF *Hexagenia* WITH 2.0 MG/LITER O_2 AND VARIED CONCENTRATIONS OF H_2S

(expressed as percentage)

Day	H_2S concentration, mg/liter					
	0.00	0.18	0.25	0.35	0.43	0.54
1	0	0	0	0	30	90
2	0	11	22	20	70	100
3	10	11	78	80	100	100
4	20	22	78	100	100	-[b]
5	0	33	89	100	100	-
7	30	78	100	-	-	-
8	40	100	-	-	-	-
9	20	100	-	-	-	-
10	60	100	-	-	-	-

[a] Emergence of nymphs from burrows.

[b] Dash after 100 indicates all dead.

Table 121. FEEDING OF *Gammarus* ON *Populus alba pyramidalis* LEAVES
AT VARIOUS LEVELS OF H_2S
(intake expressed as mg/individual/day)

H_2S concentration, mg/liter	Test 1[a]	Test 2[a]	Test 3[b]
0.0	0.677	0.604	0.431
0.010	-	-	0.381
0.013	0.550	-	-
0.016	-	0.680	-
0.020	-	-	0.367
0.028	0.489	-	-
0.031	-	-	0.340
0.033	-	0.428	-
0.039	-	-	0.244
0.047	-	0.196	-
0.050	-	-	0.135

[a] 6.0 mg/liter O_2, 15 C, and pH 7.51.
[b] 5.8 mg/liter O_2, 10 C, and pH 7.52.

Table 122. 96-HOUR LC50 VALUES OF H_2S FOR SIX INVERTEBRATES COLLECTED AT DIFFERENT SEASONS

Species	pH	O_2, mg/1	Temperature, C	Days	96-hr LC50, mg/1 H_2S	Season
Asellus	7.5	2.0	15.2	4	1.07	Winter
Crangonyx	7.4	2.0	14.9	4	0.84	Winter
Gammarus	7.5	5.9	15.0	4	0.059	Winter
Baetis	7.6	6.2	14.8	4	0.020[a]	Summer
Ephemera	7.4	1.9	15.0	4	0.316	Winter
Hexagenia	7.7	2.0	15.0	4	0.111[b]	Summer
	7.7	2.0	15.0	4	0.151	Winter
	7.4	2.0	15.0	4	0.365	Winter

[a] Mean of two tests.

[b] Mean of seven tests - July collection.

mayflies. The most sensitive were the flowing stream forms with a 96-hr LC50 that was 2-6% that of the most resistant forms. In general, as might be expected from flowing stream forms, the degree of resistance was closely related to the probable occurrence of low oxygen or high H_2S in their normal habitat.

CHRONIC TESTS

Hexagenia limbata (Serville)

Hexagenia nymphs inhabit U-shaped burrows dug in fine bottom sediment. Eriksen[25] demonstrated that the stratum of water available to 20-35 mm nymphs was the 6-7 mm layer above the mud-water interface. Earlier nymphal stages (<1 mm at hatch) have a correspondingly reduced water stratum available. The work reported here was designed to test the effects of chronic exposure to H_2S under general habitat conditions as close as possible to optimum for the species. Acute tests were run for comparison.

Experimental Design--Nymphs for the study were collected from the Crystal Beach area of Rainy Lake near Ranier, Minnesota on May 19, 1973 at 2 to 4 ft. Mud with nymphs still in the burrows was placed in a screen-bottomed box, suspended in lake water, and the mud gently washed out. Nymphs were transferred to the laboratory in lake water. The mean length of the nymphs from the tip of the rostrum to the tip of the abdomen was 1.77 cm. Prior to testing nymphs were held in glass aquaria (50 x 25 x 20 cm) with a water depth of 16 cm. Three cm of mud from the same area in which the nymphs were collected was placed on the bottom. The 200 nymphs held in each aquarium formed burrows within a few hours after placement. Water at 18 C, pH 7.8, and saturated with O_2 was passed through each aquarium at the rate of 500 ml/min during pretest holding period. The nymphs were fed a finely ground suspension of lettuce twice daily and recently hatched brine shrimp once each day.

The acute test was conducted in flow-through apparatus with five toxicant concentrations and one control as described by Colby and Smith[1] and Adelman and Smith.[2] Toxicant was added as sodium sulfide from stock solution by the "dipping bird" dispenser. Test chambers for acute studies were identical with those used for laboratory maintenance of the nymphs. Water flow through each chamber was 300 ml/min and a light cycle of 12 hr of light and 12 hr of darkness was maintained. Nymphs were not fed during the first 96 hr of each test but thereafter were fed on the same schedule as the laboratory stock. Analyses were made three times each day for H_2S and once each day for pH, temperature, dissolved O_2, and total alkalinity. Water samples for the determination of H_2S, pH, temperature, and dissolved O_2 were siphoned from near the mud-water interface of each chamber. H_2S samples were fixed immediately to insure that the exact concentration assumed to be bathing the nymphs in the burrows was determined.

Chronic tests employed two diluters modified from that of Brungs and Mount[4] and the same test chambers described for nymph maintenance. Each diluter served four concentrations and one control. H_2S concentrations from one diluter were alternated randomly with those of the other to form a continuous series. Temperature and pH were controlled and water was saturated with O_2 in the head tanks. Chemical analyses were made in the same manner as in the acute tests. H_2S, pH, and temperature were determined three times per week and dissolved O_2 once weekly. The feeding schedule was that used for laboratory maintenance. Chronic treatment of nymphs was started on July 9, 1973 and terminated 138 days later. Mortality of nymphs and emergence of subimagos to adults were determined daily.

Acute Toxicity--Acute tests were run in connection with chronic tests for critical comparison. H_2S concentrations in these tests ranged from 0.0251-0.4723 mg/liter with one control, temperature from 17.8-18.3 C, pH from 7.67-7.99, and O_2 from 4.53-6.63 mg/liter in the various chambers (Table 123). Total alkalinity as $CaCO_3$ was constant at 235 mg/liter.

Table 123. CHARACTERISTICS OF TEST DURING EXPOSURE OF *Hexagenia* IN THE ACUTE TEST

	Test chamber					
Item	1	5	4	3	2	6
$\bar{x}$ H_2S (mg/1)	0	0.0251	0.0466	0.1078	0.2890	0.4723
Std. Dev.	0	0.0123	0.0159	0.0227	0.0230	0.0151
$\bar{x}$ pH	7.99	7.90	7.90	7.84	7.73	7.67
$\bar{x}$ temperature (C)	18.3	18.2	18.2	18.1	17.8	17.9
$\bar{x}$ dissolved O_2 (mg/1)	6.63	6.03	6.22	5.81	4.94	4.53
$\bar{x}$ total alkalinity (mg/1)	235	235	235	235	235	235

A total of 10 individuals was placed in each test chamber. The LC50 concentration of H_2S dropped from 0.312 mg/liter at 48 hr to 0.165 mg/liter at 96 hr and at 12 days was 0.060 mg/liter (Table 124). Percentage survival dropped to 80% in the lowest treatment after 6 days but in all others after the first or second day. Survival in control over the entire period was 70%.

Chronic tests--The test conditions in the two chronic bioassays (Table 125) included one control and four H_2S concentrations ranging from 0.0-0.0762 mg/liter. Temperature, pH, and dissolved O_2 were similar to those in acute tests. A total of 10-13 nymphs were placed in each tank (Table 126). Mortality was low (0-9%) in all concentrations lower than 0.0290 mg/liter H_2S. At this concentration mortality was 37% and at 0.0762 mg/liter H_2S none survived. Emergence of subimagos varied from 30-75% at levels below 0.0348 mg/liter H_2S. At this concentration and higher no emergence occurred.

There was a small but non-significant reduction in length of subimagos as the H_2S concentration increased in each experiment. Nymphs exposed to 0.0152 mg/liter H_2S were 6% shorter and at 0.0129 and 0.0290 mg/liter H_2S were 3% shorter than the controls.

Summary--On the basis of nymphal survival and the percentage which emerged as subimagos in the chronic tests, it is apparent that concentrations up to 0.0152 mg/liter H_2S in the Diluter 1 test and 0.0129 mg/liter in the Diluter 2 test are not different from the controls (Table 126).

With a 96-hr LC50 of 0.165 mg/liter H_2S and the highest safe level of 0.0152 mg/liter, the application factor (.0152/.165) would be .09. A ratio based on the 12-day LC50, highest safe concentration, and application factors is made between the *Hexagenia* and data from the amphipod, *Gammarus* (Table 127). It is apparent that although there are wide differences in the 96-hr LC50, 12-day LC50, and highest chronic safe

Table 124. SURVIVAL OF NYMPHS AND CALCULATED LC50'S OF H_2S TO *Hexagenia* ON SUCCEEDING DAYS DURING THE ACUTE TEST[a]

(expressed as percentage)

	LC50,	H_2S concentration, mg/1					
Day	mg/1 H_2S	0.0	0.0251	0.0466	0.1078	0.2890	0.4723
1	-	100	100	100	100	100	100
2	0.312	100	100	90	100	60	0
3	0.185	100	100	90	90	30	-
4	0.165	100	100	80	80	20	-
5	0.135	100	100	80	80	0	-
6	0.134	100	100	60	80	-	-
7	0.120	100	90	50	70	-	-
8	0.110	100	90	50	60	-	-
9	0.050	100	80	50	40	-	-
10	0.072	100	80	50	20	-	-
11	0.072	100	80	50	20	-	-
12	0.060	100	80	50	0	-	-

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[a] Survival values have been corrected on the basis of survival in the control tank (Abbott[9]).

Table 125. TEST CONDITIONS DURING CHRONIC EXPOSURE OF *Hexagenia*

Item	Test chamber - Diluter 1				
	3	2	1	5	4
$\bar{x}$ H_2S concentration (mg/l)	-	0.0011	0.0060	0.0152	0.0348
Std. Dev.	-	0.0009	0.0022	0.0050	0.0105
$\bar{x}$ pH	7.9	7.9	8.0	8.0	8.0
$\bar{x}$ temperature (C)	17.7	17.8	18.0	17.7	17.8
$\bar{x}$ dissolved O_2 (mg/l)	7.53	7.65	7.37	6.86	5.52

	Test chamber - Diluter 2				
	8	10	9	7	6
$\bar{x}$ H_2S concentration (mg/l)	-	0.0042	0.0129	0.0290	0.0762
Std. Dev.	-	0.0017	0.0060	0.0096	0.0153
$\bar{x}$ pH	7.8	7.9	7.9	7.9	8.2
$\bar{x}$ temperature (C)	17.5	17.8	17.6	17.7	17.9
$\bar{x}$ dissolved O_2 (mg/l)	7.34	7.11	6.84	6.06	4.75

Table 126. SUMMARY OF SURVIVAL OF *Hexagenia* NYMPHS AND EMERGENCE OF SUBIMAGOS DURING THE CHRONIC TEST

	Test chamber									
	Diluter 1					Diluter 2				
Item	3	2	1	5	4	8	10	9	7	6
H_2S (mg/l)	0	0.0011	0.0060	0.0152	0.0348	0	0.0042	0.0129	0.0290	0.0762
Number of nymphs	12	13	10	11	11	13	13	12	11	11
Percentage deaths	8	8	0	9	36	8	0	0	37	100
Percentage subimagos emerged	50	38	30	36	0	54	62	75	36	0
Percentage emerged or survived in 138 days	92	92	100	91	64	92	100	100	73	0

Table 127. RELATIONSHIPS BETWEEN LC50, CHRONIC SAFE, AND APPLICATION FACTOR VALUES FOR *Hexagenia* AND *Gammarus*

Item	*Hexagenia*	*Gammarus*
96-hr LC50 (mg/l H_2S)	0.165	0.022
12-day LC50 (mg/l H_2S)	0.060	0.011
Highest safe level (mg/l H_2S)	0.015	0.002
Application factor based on 96-hr LC50	.09	.09
Application factor based on 12-day LC50	.25	.20

level, the differences in application factors are quite small.

Gammarus pseudolimnaeus Bousfield

To determine the long-term effect of exposure to sublethal levels of H_2S, four tests were run on *Gammarus* from 65 to 105 days. This period permitted a minimum of one reproductive cycle.

Experimental Design--*Gammarus* for the experiments were secured from two spring-fed streams; Valley Creek flowing into the St. Croix River near Afton, Minnesota and an unnamed stream flowing into the St. Croix River near Marine on St. Croix, Minnesota. Collections were made by placing a screen on the bottom and gently stirring bottom vegetation to dislodge the organisms. Collections were made on November 20, February 16, July 27, and December 29 when stream temperatures were 7, 6, 12, and 3 C, respectively.

Acute tests were made prior to the start of each chronic test to determine the 96-hr LC50 of H_2S to the test organisms. Chronic tests were then started with individuals from the same stock and continued for 65 to 105 days. Specimens were raised to a temperature of 18 C over a period of 5 to 7 days and then held for 10 days prior to the start of acute and chronic tests. During the acclimation and chronic test periods they were fed leaves of *Populis alba pyramidalis*. Leaves were presoaked in flowing laboratory water for 2 wk before they were used as food.

Acute tests were made in the apparatus described by Adelman and Smith[2] employing glass test chambers 50 x 25 x 20 cm. Water depth was 16 cm and total volume 20 liters. Flow rate through the chambers was 300 ml/min. H_2S concentrations at the center of each chamber were determined three times a day during the test. Acute tests were run with five levels of H_2S and one control.

Chronic exposures on new individuals were made in the same chambers as were used for acute tests with a diluter modified as described by Brungs and Mount.[4] Four H_2S concentrations and one control were employed with a flow-through rate of 125 ml/min. The concentration was analysed three times per week. Gammarus were fed leaves as described above with a sufficient number placed in each tank to provide both food and cover. Tanks were illuminated with 61-cm Duratest Vitalite fluorescent lamps providing 50 ft-candles at the water surface. Photoperiod was 16 hr of light and 8 hr of darkness. The pH and temperature were maintained by methods described by Oseid and Smith.[18] At the start of each chronic experiment, except test 3, 20 coupled pairs were placed in each chamber. In test 3, 40 individuals were used without reference to sex. At the completion of each test after 65, 96, and 105 days, formalin was added to each tank to kill and preserve the organisms. They were counted and measured within 1 month and the entire group was weighed after centrifuging to remove adherent water.

Effect of H_2S--Acute tests were run with H_2S concentrations ranging from 0.008 to 0.093 mg/liter (Table 128). Temperature varied from 17.8-18.1 C, oxygen from 5.8-7.4 mg/liter, and pH from 7.7-7.9. Total alkalinity ranged from 222-232 mg/liter $CaCO_3$. The total length of organisms exclusive of antennae was 0.7-1.2 cm. For the successive tests the 96-hr LC50's were 0.022, 0.022, 0.024, and 0.021 mg/liter H_2S. A single threshold test run through 18 days gave an LTC value of 0.011 mg/liter H_2S.

H_2S concentration in the four chronic tests varied from 0.0007 to 0.0192 mg/liter (Table 129). Temperature in three tests varied from 17.1-17.8 C and in one test was 18.0-18.2 C. Dissolved O_2 ranged from 7.4-8.9 mg/liter. Survival at the higher levels was as low as 4% of the control and as high as 57% in another test. The duration of the exposure did not seem to have a direct relationship to the survival totals but the maximum number was assumed to be controlled by chamber size which was constant. Length-frequency distribution of the organisms surviving at

Table 128. ACUTE TOXICITY OF H_2S TO *Gammarus* USED IN CHRONIC STUDIES

Test	Range of H_2S conc.,[a] mg/l	Temperature, C	pH	96-hr LC50, mg/l H_2S	Threshold LTC,[b] mg/l H_2S
1	0.012-0.093	17.9	7.7	0.022	-
2	0.012-0.061	17.8	7.9	0.022	-
3	0.009-0.044	18.0	7.8	0.024	-
4	0.008-0.054	18.1	7.9	0.021	0.011

[a] Set up in logarithmic series.
[b] 18-day duration.

Table 129. CONDITIONS DURING CHRONIC TESTS OF *Gammarus* WITH H_2S[a]

	Test 1 (65 days)				
$\bar{x}$ H_2S conc. (mg/l)[b]	Control	0.0007	0.0019	0.0031	0.0128
Std. Dev.	-	0.0007	0.0013	0.0021	0.0075
$\bar{x}$ Temperature (C)	17.2	17.1	17.1	17.3	17.2
$\bar{x}$ pH	7.80	7.81	7.83	7.76	7.86
No. survivors	446	548	504	117	19
Weight survivors (g)[c]	1.961	2.349	2.648	1.083	0.091
	Test 2 (105 days)				
$\bar{x}$ H_2S conc. (mg/l)	Control	0.0007	0.0013	0.0029	0.0071
Std. Dev.	-	0.0012	0.0017	0.0027	0.0044
$\bar{x}$ Temperature (C)	17.4	17.3	17.4	17.6	17.5
$\bar{x}$ pH	7.66	7.65	7.67	7.60	7.69
No. survivors	477	380	793	458	363
Weight survivors (g)[c]	3.320	1.628	3.402	2.254	2.390
	Test 3 (95 days)				
$\bar{x}$ H_2S conc. (mg/l)	Control	0.0012	0.0032	0.0064	0.0153
Std. Dev.	-	0.0013	0.0024	0.0032	0.0051
$\bar{x}$ Temperature (C)	17.7	17.6	17.7	17.8	17.7
$\bar{x}$ pH	7.83	7.82	7.85	7.80	7.85
No. survivors	229	432	374	241	19
Weight survivors (g)[c]	0.932	1.361	1.179	0.914	0.302

Table 129 (continued). CONDITIONS DURING CHRONIC TESTS OF *Gammarus* WITH H_2S[a]

	Test 4 (65 days)				
$\bar{x}$ H_2S conc. (mg/l)	Control	0.0012	0.0026	0.0076	0.0192
Std. Dev.	-	0.0006	0.0008	0.0026	0.0062
$\bar{x}$ Temperature (C)	18.1	18.1	18.2	18.0	18.0
$\bar{x}$ pH	7.68	7.70	7.75	7.78	7.71
No. survivors	581	421	53	607	336
Weight survivors (g)[c]	2.478	2.150	0.367	2.778	1.174

[a] Dissolved O_2 range 7.4-8.9 mg/liter.

[b] Standard Methods of Water Analysis[6] - colorimeter used instead of tube comparison.

[c] Total weight of all survivors.

the end of the experiment was roughly the same for the four tests (Figure 8). The distribution within a single test was essentially the same except that total numbers in each size class varied with the H_2S level.

Summary--The results presented above suggest that at concentrations of H_2S below 0.002 mg/liter there was increased reproduction or survival in some tests but there was no consistent effect on mean weight of individuals. At levels in excess of 0.002 mg/liter there was a reduction in numbers and a consequent reduction in total weight of the test group. Treatments between 0.0013 and 0.019 mg/liter showed a mean reduction of 71% in numbers and 76% in total weight when compared to the controls. On the assumption that 0.002 mg/liter H_2S is the maximum safe level for Gammarus and the mean 96-hr LC50 of 0.022 mg/liter H_2S is employed, an application factor of .10 would apply. It is apparent from these data that the sensitivity of Gammarus is at approximately the same level as that of the brook trout. Although the expectancy of any significant level of H_2S in a flowing stream which is normally inhabited by Gammarus remains to be demonstrated, the extremely low level which is toxic might well be attained in bottom soil or in decaying detritus. Field studies on this point would be useful.

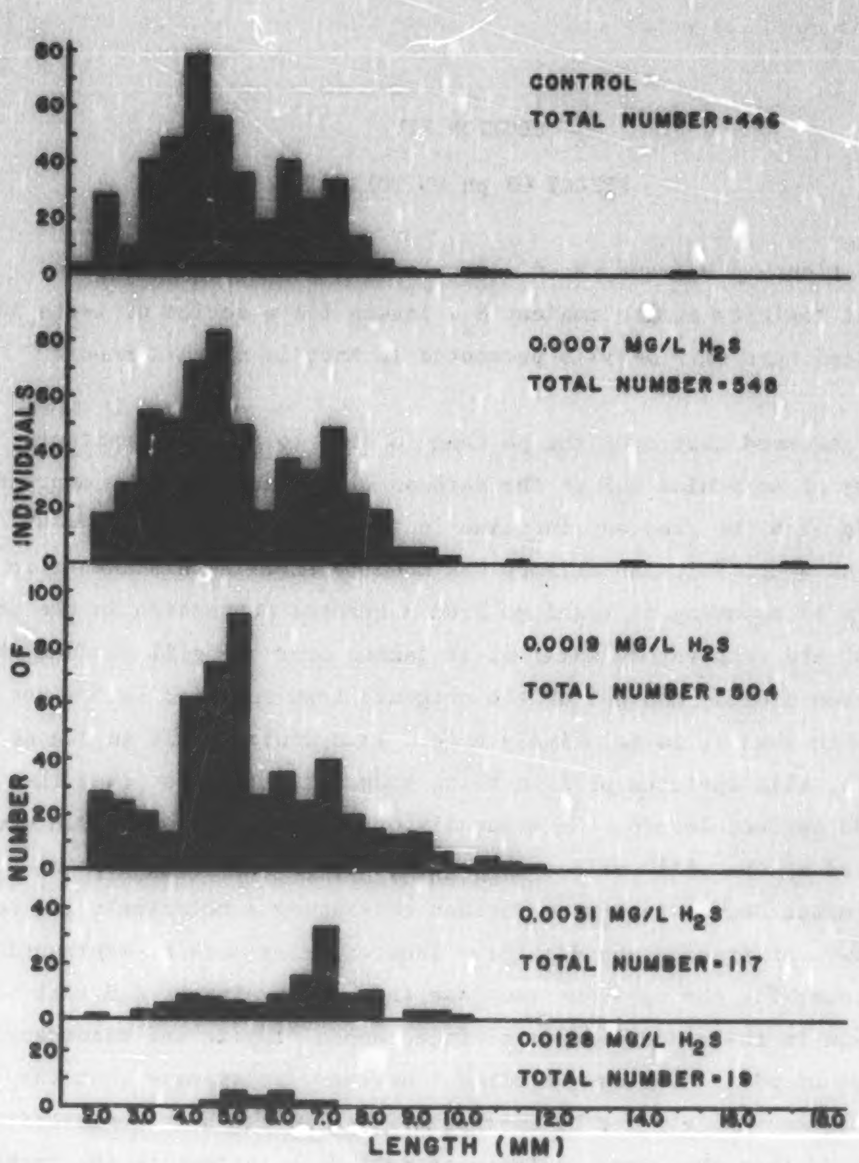

Figure 8. Length-frequency distribution of Gammarus after 65 days of exposure to varied concentrations of H_2S.

SECTION XIV

EFFECT OF pH ON TOXICITY

The relationship between pH of various sulfide test solutions and apparent toxicity of the ambient H_2S levels for a series of tests is summarized here with details presented in Part II of this report.

It was observed that over the pH range of 6.5 to 8.7 the apparent toxicity of molecular H_2S to the fathead minnow increases by approximately fourfold with the greatest increase in toxicity occurring above pH values of about 7.1. An attempt was made to explain this change in toxicity by assuming it resulted from a greater depression in the pH value of the respiratory water as it passes over the gill surface when the carbon dioxide content of the original test solution is low compared with when it is relatively high. This would result in the actual pH at the gill surfaces of fish being substantially lower than the measured ambient levels. Consequently, the concentration of H_2S would be increased at the gill surface as a result of the HS^- ions forming H_2S to a greater degree in test solutions containing a relatively low concentration of free carbon dioxide. Theoretically such an explanation may account for the apparent increase in the toxicity of H_2S with a reduction in the concentration of free carbon dioxide and accompanying increase of pH in the test solution. However, it appears that the above explanation may not be appropriate and it has been demonstrated in Part II that the acute toxicity of sulfide solutions to the fathead minnow is not entirely related to the ambient concentration of molecular H_2S but is linearly related to the ambient concentration of dissolved sulfide (i.e., H_2S plus HS^-). It is proposed that the acute toxicity of sulfide solutions of relatively low pH values results mainly from the penetration of the gills by molecular H_2S. But the increased apparent toxicity of molecular H_2S with increasing pH may result from a greater contribution in the penetration of the gills by the HS^- ion and because of the effect which the internal pH, as related to ambient CO_2 tensions, would have on the blood and intracellular sulfide equilibrium.

SECTION XV

DISCUSSION

DIFFERENCE IN ACUTE SENSITIVITY BETWEEN SPECIES

A wide range of acute sensitivity to H_2S exists between various fish species (Table 130). At comparable temperatures, LTC values for juvenile fish ranged from 0.0087 mg/liter H_2S at 12 C in 17 days with rainbow trout to 0.0840 mg/liter H_2S at 14 C for goldfish in 11 days. Other fish species varied between these extremes. The maximum difference between species was approximately fourfold for comparable temperatures and life history stage. However, temperature and life history stage have a large influence on response of individual species as will be discussed below.

DIFFERENCES IN ACUTE TOXICITY OF VARIOUS LIFE HISTORY STAGES

The acute sensitivity of various life history stages in the same species varies with fry being the most sensitive, except in the goldfish where eggs are the most sensitive (Table 131). The difference in sensitivity between the most and least sensitive stage was approximately three- to elevenfold. As fish increase in age beyond the fry stage, they become more resistant to the acute effects of H_2S. Presumably this increase is related to differences in metabolic rates.

These findings suggest that no prediction of the relative sensitivity of various stages can be projected for untested species. While temperatures of tests varied between stages in some cases, they were close to conditions usually encountered by the fish in nature.

Table 130. ACUTE TOXICITY OF H_2S TO SEVEN SPECIES OF FISH

(expressed as mg/liter)

				Original values				Corrected values[a]				
		Number of	Temp.,	96-hr LC50		LTC		96-hr LC50		LTC		
Species	Stage	tests	C	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Days
Fathead minnow[b]	Egg	6	24	.0350	.0190-.0610	.0345	.0190-.0595	.0536	.0291-.0935	.0529	.0291-.0912	4-8
Duluth stock	Fry	3	24	.0070	.0066-.0075	.0061	.0057-.0066	.0107	.0101-.0115	.0093	.0087-.0101	6
	Juvenile	1	20	.0160	-	-	-	.0238	-	-	-	-
	"	3	22	.0162	.0127-.0180	.0120	.0109-.0130	.0246	.0193-.0273	.0182	.0166-.0198	8-10
Field stock	Juvenile	4	6	.5150	.4600-.5800	-	-	.7089	.6332-.7983	-	-	-
	"	1	10	.1500	-	-	-	.2041	-	-	-	-
	"	1	15	.0570	-	-	-	.0814	-	-	-	-
	"	7	20	.0360	.0320-.0390	-	-	.0545	.0484-.0590	-	-	-
	"	6	24	.0212	.0152-.0280	.0189	.0143-.0220	.0324	.0232-.0428	.0289	.0219-.0336	7-9

Table 130 (continued). ACUTE TOXICITY OF H_2S TO SEVEN SPECIES OF FISH

(expressed as mg/liter)

				Original values				Corrected values[a]				
		Number of	Temp.,	96-hr LC50		LTC		96-hr LC50		LTC		
Species	Stage	tests	C	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Days
Goldfish	Egg	1	22	.0220	-	-	-	.0321	-	-	-	-
	Fry	1	22	.0250	-	-	-	.0370	-	-	-	-
	Juvenile	21	14	.1450	.1120–.1950	.0840	.0660–.1200	.2002	.1546–.2692	.1160	.0911–.1656	11
	"	21	20	.0830	.0680–.1150	.0710	.0520–.0840	.1201	.0984–.1664	.1027	.0752–.1215	11
	"	21	26	.0630	.0500–.0840	.0600	.0460–.0840	.0935	.0742–.1247	.0891	.0683–.1247	11
Sucker	Egg[c]	1	15	.0280	-	-	-	.0383	-	-	-	-
	"[c]	1	13	-	-	.0190	-	-	-	.0268	-	12
	Fry[c]	3	15	.0210	.0130–.0260	-	-	.0287	.0178–.0356	-	-	-
	Juvenile	4	20	.0219	.0185–.0290	-	-	.0330	.0279–.0437	-	-	-

Table 130 (continued). ACUTE TOXICITY OF H_2S TO SEVEN SPECIES OF FISH

(expressed as mg/liter)

		Number		Original values				Corrected values[a]				
		of	Temp.,	96-hr LC50		LTC		96-hr LC50		LTC		
Species	Stage	tests	C	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Days
Bluegill	Egg	2	22	.0144[d]	.0125-.0162	-	-	.0219	.0190-.0246	-	-	-
	Sac fry	1	22	-	-	.0169	-	-	-	.0253	-	9
	Swim-up fry	1	22	.0086	-	.0084	-	.0131	-	.0128	-	8
	Juvenile	5	20	.0316	.0290-.0325	.0318	.0310-.0325	.0478	.0438-.0491	.0481	.0468-.0491	8-10
	Adult	7	20	.0297	.0198-.0375	-	-	.0448	.0298-.0565	-	-	-
Walleye	Egg[c]	2	12	.0520	-	.0290	.0220-.0360	.0720	-	.0402	.0305-.0499	10-19
	" [c]	2	15	.0805	.0740-.0870	-	-	.1103	.1014-.1192	-	-	-
	Fry[c]	1	15	.0070	-	-	-	.0096	-	-	-	-
	Juvenile	4	15	.0193	.0166-.0214	-	-	.0282	.0242-.0312	-	-	-

Table 130 (continues). ACUTE TOXICITY OF H_2S TO SEVEN SPECIES OF FISH

(expressed as mg/liter)

				Original values				Corrected values[a]				
		Number		96-hr LC50		LTC		96-hr LC50		LTC		
		of	Temp.,									
Species	Stage	tests	C	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Days
Rainbow trout	Egg	1	13	-	-	.0154	-	-	-	.0215	-	29
	Fry	1	13	-	-	.0056	-	-	-	.0077	-	20
	Juvenile	1	12	.0130	-	.0087	-	.0181	-	.0121	-	17
	"	1	15	.0125	-	-	-	.0179	-	-	-	-
Brook trout	Egg	2	9	-	-	.0760	.0738-.0783	-	-	.1035	.1005-.1066	10-13
	"	2	14	-	-	.0500	.0485-.0516	-	-	.0697	.0676-.0719	8-9
	Sac fry	2	9	.0210	.0206-.0214	.0160	.0160-.0161	.0286	.0280-.0291	.0218	.0218-.0219	10
	" "	2	14	.0148	.0138-.0158	.0120	.0117-.0124	.0204	.0190-.0218	.0165	.0161-.0171	10
	Swim-up	2	9	.0233	.0232-.0234	.0223	.0220-.0226	.0318	.0316-.0319	.0304	.0300 .0308	10

Table 130 (continued). ACUTE TOXICITY OF H_2S TO SEVEN SPECIES OF FISH
(expressed as mg/liter)

				Original values				Corrected values[a]				
		Number of	Temp.,	96-hr LC50		LTC		96-hr LC50		LTC		
Species	Stage	tests	C	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Days
Brook trout	Swim-up fry	2	14	.0216	.0215-.0217	.0186	.0186-.0187	.0302	.0301-.0304	.0260	.0260-.0262	9
	Juve-nile	3	8	.0266	.0245-.0297	.0197	.0187-.0212	.0358	.0330-.0400	.0265	.0252-.0286	11-12
	"	2	11	.0230	.0228-.0233	.0178	.0171-.0184	.0313	.0310-.0317	.0242	.0232-.0250	9
	"	3	14	.0183	.0156-.0219	.0155	.0153-.0156	.0263	.0224-.0315	.0223	.0220-.0224	6-11
	"	4	16	.0168	.0163-.0173	.0138	.0129-.0146	.0240	.0233-.0247	.0197	.0184-.0209	8-10
	"	2	19	.0168	.0168	-	-	.0244	.0244	-	-	-
	"	2	21	.0178	.0177-.0179	.0078	.0078	.0264	.0262-.0265	.0116	.0116	10-12

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[a] Values derived from Pomeroy constants corrected to new values from ionization constants worked out in this study.

[b] Does not include 40 sets of acute tests on fathead minnows to determine seasonal and geographical variation.

[c] Data from Smith and Oseid.[18]

[d] LC50 at hatch time of 66 and 77 hr.

Table 131. MEDIAN TOLERANCE LIMITS (LC50) TO H_2S OF EGGS, FRY, AND JUVENILES OF VARIOUS SPECIES ($mg/liter\ H_2S$ - duration days in parentheses)[a]

		Original values				Corrected values[b]			
Species	Temp., C	Egg	Sac fry	Swim-up fry	Juvenile	Egg	Sac fry	Swim-up fry	Juvenile
Brook trout	9	0.0760	0.0160	0.0223	0.0197	0.1035	0.0218	0.0304	0.0265
		(10-13)	(10)	(10)	(11-12)				
	14	0.0500	0.0120	0.0186	0.0155	0.0697	0.0165	0.0260	0.0223
		(8-9)	(10)	(9)	(6-11)				
Rainbow trout	13	0.0154	0.0056	-	0.0087	0.0215	0.0077	-	0.0121
		(29)	(20)		(17)				
Sucker	15	0.0280[c]	0.0210[c]	-	0.0219	0.0383[c]	0.0287[c]	-	0.0330
		(4)	(4)		(4)				
Northern pike[d]	13	0.037	0.026	-	-	0.052	0.036	-	-
		(4)	(4)						
Fathead	24	0.0345	0.0061	-	0.0120[e]	0.0529	0.0093	-	0.0182[e]
		(4-8)	(6)		(8-10)				

Table 131 (continued). **MEDIAN TOLERANCE LIMITS (LC50) TO H_2S OF EGGS, FRY, AND JUVENILES OF VARIOUS SPECIES**

(mg/liter H_2S - duration days in parentheses)[a]

		Original values				Corrected values[b]			
Species	Temp., C	Egg	Sac fry	Swim-up fry	Juvenile	Egg	Sac fry	Swim-up fry	Juvenile
Goldfish	22	0.0220 (4)	0.0250 (4)	-	0.0830[f] (4)	0.0321	0.0370	-	0.1201[f]
Walleye	15	0.0805[c] (4)	0.0070[c] (4)	-	0.0193 (4)	0.1103[c]	0.0096[c]	-	0.0282
Bluegill	22	0.0144 (3)	0.0169 (9)	0.0084 (8)	0.0318[f] (8-10)	0.0219	0.0253	0.0128	0.0481[f]

[a] Values in excess of 4 days are LTC values.

[b] Corrected values represent change from Pomeroy ionization constants to new constants worked out in this study.

[c] Smith and Oseid.[18]

[d] Adelman and Smith.[2]

[e] At 22 C.

[f] At 20 C.

FACTORS INFLUENCING ACUTE SENSITIVITY OF FISH

Temperature and acclimation influence the acute sensitivity of fish to H_2S. The most influential factor is temperature. Sensitivity in fathead minnows varied in 96-hr LC50 values from 0.5150 mg/liter H_2S at 6.1 C to 0.0212 mg/liter H_2S at 24 C, approximately a twenty-fivefold difference. In goldfish tested at 14.1 and 26 C, the difference was approximately 2.5 times. While the differences are not as marked as in the fathead, extrapolation of the temperature values approximates the same slope. Brook trout tested between 8.2 and 21 C did not show as marked a difference as fathead minnows or goldfish. When threshold LC50 concentrations are compared, the differences are not as marked. In a range of 12 C in goldfish the difference is approximately 1.5 times. In brook trout, however, the threshold value with a 13 C difference in temperature changes sensitivity approximately threefold. The implications of these data for natural distribution of the various organisms suggest that the cyprinids can stand for short periods at least extreme concentrations at low temperatures while the other species have a much lower degree of increased tolerance at the low temperatures. The implications for year round standard setting are evident.

The season of the year at which wild fish are subjected to H_2S appears to have some influence on their resistance. Fathead minnows taken late in the winter appear to have somewhat less resistance at comparable temperatures than those taken during the open water season.

Another factor influencing sensitivity is acclimation. It is apparent from data collected on both fathead minnows and bluegills that a certain degree of acclimation to H_2S can take place provided that initial exposure is not in the acutely lethal range. This finding suggests that a continuous low exposure, although it may have effects on long-term survival, growth, reproduction etc., does make the organisms more tolerant (Tables 51, 52).

ACUTE TOXICITY TO INVERTEBRATES

The sensitivity of invertebrate organisms varied from an LC50 of 1.070 mg/liter H_2S in *Asellus* to 0.020 mg/liter H_2S in *Baetis* (Table 132). The two species (*Gammarus* and *Baetis*) which inhabit cold and well-aerated flowing waters were the most sensitive. Conversely, the two species (*Asellus* and *Crangonyx*) which are typically found where anoxic conditions are common showed the most resistance. The coldwater species were in the same general area of sensitivity as the coldwater fish. The tests on invertebrates were run under conditions assumed to be preferential both as to habitat and to temperature for the particular period of the year. The burrowing forms, as would be expected, are more tolerant than those which live on the surface.

RESPONSE OF FISH AND INVERTEBRATES TO LONG-TERM EXPOSURE TO H_2S

The H_2S concentrations at which growth, reproduction, survival, and performance were tested indicated that in most species the no-effect level was much lower than the LTC and in some species any measurable level of H_2S had some adverse effect (Table 133). The length of exposure in the chronic tests extended to 825 days in one case but in most tests for a shorter period of time. Growth rate in most species of fish was found to be as good or better an index to adverse effect as survival or reproduction. Exceptions were found in bluegills and brook trout in which reproductive success was the most sensitive indicator of adverse effect. In bluegills and brook trout the adverse effect was related to behavior which limited sexual activity. When fish which had poor spawning performance or no spawning performance at all were placed in fresh water, they immediately proceeded to go through the spawning act and deposit eggs. Non-spawning fish in high levels of H_2S which were examined showed no apparent reduction in eggs contained or in other anatomical features. Brook trout were inhibited completely from spawning activity at higher levels but like the bluegill spawned effectively when transferred into non-toxic medium. However, brook trout at all levels of exposure showed a reduced production of eggs. The relative influence of

Table 132. ACUTE TOXICITY OF H_2S TO EIGHT SPECIES OF INVERTEBRATES

Species	Number of tests	Temperature, C	LC50 range, mg/l H_2S	Duration, days	Season	Preferred[d] 96-hr LC50, mg/l H_2S Original value	Preferred[d] 96-hr LC50, mg/l H_2S Corrected value[e]	pH
Asellus[a,b]	4	15.2	1.010-1.700	4	Winter	1.07	1.46	7.5
Crangonyx[a]	8	14.9	0.310-0.840	4	Winter	0.84	1.14	7.4
Gammarus[a]	10	15.0	0.030-0.059	4-10	Winter	0.059	0.081	7.5
	4	18.0	0.021-0.024	4	Summer & winter	0.022	0.032	7.8
Baetis[a]	2	14.8	0.020-0.026	2-4	Summer	0.020	0.028	7.6
Ephemera[a]	5	15.0	0.135-0.380	4-11	Winter	0.316	0.429	7.4
Hexagenia[a,c]	39	15.0	0.026-0.680	2-11	Summer	0.111	0.155	7.7
					Winter	0.151	0.211	7.7
	1	18.0	0.165	4	Summer	0.165	0.244	7.8
Procambarus								
Egg	1	14.2	-	4	-	>0.408	>0.568	7.7
	1	18.0	-	4	-	>0.433	>0.626	7.7
	1	21.9	-	4	-	0.370	0.557	7.7
Larvae	1	17.9	-	4	-	0.125	0.177	7.6
	1	22.0	-	4	-	0.058	0.087	7.7
Juvenile	1	14.1	-	4	-	0.147	0.204	7.7
	1	18.0	-	4	-	0.083	0.121	7.7
	1	22.1	-	4	-	0.034	0.051	7.7

Table 132 (continued). ACUTE TOXICITY OF H_2S TO EIGHT SPECIES OF INVERTEBRATES

Species	Number of tests	Temperature, C	LC50 range, mg/l H_2S	Duration, days	Season	Preferred[d] 96-hr LC50, mg/l H_2S Original value	Preferred[d] 96-hr LC50, mg/l H_2S Corrected value[e]	pH
Procambarus								
Subadult	8	18.0	-	4	-	0.093	0.137	7.8
	1	25.9	-	4	-	0.075	0.114	7.8
Adult	1	14.0	-	4	-	0.271	0.375	7.7
	1	18.1	-	4	-	0.215	0.308	7.7
	1	21.7	-	4	-	0.121	0.176	7.6
Cambarus								
Subadult	1	13.9	-	4	-	0.150	0.205	7.7
	1	18.1	-	4	-	0.108	0.157	7.7
	1	22.0	-	4	-	0.070	0.105	7.7

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[a] Data from Oseid and Smith[26].

[b] Tests conducted after varying periods of laboratory acclimation.

[c] Tests made at various seasons and test conditions account for extreme variation of range.

[d] "Preferred LC50" is level determined under conditions judged to be most appropriate to the species.

[e] Value corrected from Pomeroy constants to ionization constants worked out in this study.

Table 133. CHRONIC TOXICITY OF H_2S TO SEVEN SPECIES OF FISH AND THREE INVERTEBRATE SPECIES (expressed as mg/liter H_2S)

				Original values			Corrected values[a]			
Species	Stage at start	Duration, days	Temperature, C	Test range	No effect	Lowest effect conc.	Test range	No effect	Lowest effect conc.	Factor of effect[b]
Rainbow trout	Sac fry	100	14.7	.0018-.0131	.0032	.0075	.0026-.0192	.0047	.0110	S+G
	10-day fry	90	14.8	.0011-.0106	.0033	.0048	.0016-.0154	.0048	.0070	S+G
	50-day juveniles	50	15.0	.0011-.0102	.0059	.0102	.0016-.0148	.0085	.0148	S+G
	Green eggs	145	13.7	.0009-.0052	.0025	.0052	.0013-.0073	.0035	.0073	S
	Eyed eggs	111	13.4	.0012-.0123	.0039	.0063	.0017-.0172	.0055	.0088	S+G
Brook trout	Adult	45-75	12.9	.0055-.0128	<.0055	.0055	.0079-.0183	<.0079	.0079	R
	0.09 g fingerlings	72	13.0	.0015-.0142	.0066	.0090	.0021-.0197	.0091	.0125	G
	0.5 g fing.	120	13.0	.0015-.0130	.0067	.0090	.0020-.0178	.0092	.0124	G
Fathead minnow	Juvenile	191	20.0	.0015-.0126	.0070	.0080	.0023-.0190	.0106	.0120	S
	Juvenile	345	21.3	.0004-.0198	.0078	.0198	.0006-.0295	.0116	.0295	S+G

Table 133 (continued). CHRONIC TOXICITY OF H_2S TO SEVEN SPECIES OF FISH AND THREE INVERTEBRATE SPECIES

(expressed as mg/liter H_2S)

				Original values			Corrected values[a]			
Species	Stage at start	Duration, days	Temperature, C	Test range	No effect	Lowest effect conc.	Test range	No effect	Lowest effect conc.	Factor of effect[b]
Fathead minnow	Sac fry	84	23.0	.0006-.0102	.0052	.0078	.0009-.0152	.0077	.0116	S+G
	Juvenile	112	20.0	.0014-.0111	.0041	.0092	.0021-.0165	.0061	.0136	S
	Sac fry	297	23.0	.0004-.0068	.0033	.0068	.0006-.0101	.0049	.0101	S+G+R
	Juvenile	354	24.0	.0006-.0080	.0035	.0074	.0009-.0120	.0052	.0111	S+G+R
Goldfish	Juvenile	294	18.6[c]	.0020-.0250	.0100	.0250	.0028-.0350	.0140	.0350	G
	Adult	294	18.6[c]	.0020-.0280	.0050	.0100	.0028-.0392	.0070	.0140	R
	Eggs	430	21.5	.0070-.0240	.0070	.0090	.0101-.0346	.0101	.0130	G
Bluegill	Adult	288	20.2[c]	.0014-.0098	.0023	.0071	.0021-.0147	.0034	.0106	G
	Juvenile	826	11.8[c]	.0015-.0064	.0015[d]	.0031	.0022-.0094	.0022	.0046	G+S+R
	Juvenile	126 & 148	24.0	.0004-.0146	.0004	.0015	.0006-.0220	.0006	.0023	SE
	Adult	200	15.0	.0010-.0105	.0062	.0105	.0014-.0144	.0085	.0144	G
	Eggs	316	22.4	.0021-.0092	-	.0021	.0031-.0136	-	.0031	G+S
	Eggs	120	24.0	.0012-.0087	-	.0012	.0018-.0127	—	.0018	S

Table 133 (continued). CHRONIC TOXICITY OF H_2S TO SEVEN SPECIES OF FISH AND THREE INVERTEBRATE SPECIES

(expressed as mg/liter H_2S)

				Original values			Corrected values[a]			
Species	Stage at start	Duration, days	Temperature, C	Test range	No effect	Lowest effect conc.	Test range	No effect	Lowest effect conc.	Factor of effect[b]
Bluegill	Eggs	93	22.2	.0010-.0073	-	-	.0015-.0110	-	-	-
	Adult	97	23.6	.0007-.0078	-	.0007	.0010-.0118	-	.0010	R
Walleye	Juvenile	225	17.8	.0013-.0051	.0031	.0051	.0018-.0071	.0043	.0071	S
	Juvenile	234	19.6	.0031-.0118	.0057	.0118	.0043-.0165	.0080	.0165	S
Gammarus	Adult	65	17.2	.0007-.0128	.0019	.0031	.0010-.0189	.0028	.0046	R+S
	Adult	105	17.4	.0007-.0071	.0013	.0029	.0010-.0100	.0018	.0041	R+S
	Adult	95	17.7	.0012-.0153	.0012	.0032	.0018-.0223	.0018	.0047	R+S
	Adult	65	18.1	.0012-.0192	-	.0012	.0018-.0284	-	.0018	R+S
Hexagenia	Nymphs	138	17.8	.0011-.0762	.0152	.0290	.0016-.1128	.0225	.0429	S
Procambarus	Eggs	447	18.1	.0041-.0183	.0041	.0086	.0060-.0267	.0060	.0126	S
	Eggs	112	15.9	.0048-.0158	.0097	.0127	.0068-.0222	.0136	.0179	S

Table 133 (continued). CHRONIC TOXICITY OF H_2S TO SEVEN SPECIES OF FISH AND THREE INVERTEBRATE SPECIES

(expressed as mg/liter H_2S)

				Original values			Corrected values[a]			
Species	Stage at start	Duration, days	Temperature, C	Test range	No effect	Lowest effect conc.	Test range	No effect	Lowest effect conc.	Factor of effect[b]
Procambarus	Juvenile	196	14.3	.0044-.0199	.0044	.0078	.0062-.0281	.0062	.0110	G
	Juvenile	196	20.6	.0044-.0172	.0044	.0088	.0066-.0256	.0066	.0131	G

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[a] Values corrected from Pomeroy constants to constants worked out in this study.

[b] S = Survival, G = Growth, R = Reproduction based on survival of offspring, SE = Swimming Endurance.

[c] Mean of seasonal variation.

[d] Reproduction inhibited at 0.0015 mg/liter H_2S.

H_2S in reducing egg potential and the effect on behavior which reduced actual deposition was not determined definitively. Fathead minnows running through two generations showed no highly significant difference in reproductive capacity at the various H_2S levels tested, but growth was a sensitive index.

The chronic toxicity of H_2S to invertebrates was determined from Gammarus, Hexagenia, and Procambarus. In Gammarus the no-effect level was 0.0013 mg/liter at 17.4 C in 105 days and in Hexagenia was 0.0152 mg/liter at 17.8 C in 138 days. Procambarus at 18.1 C showed an adverse effect at 0.0086 mg/liter in 447 days. Reproduction was not affected at 0.0090 mg/liter.

USE OF APPLICATION FACTORS

The present study has afforded an opportunity to compare the use of application factors derived from no-effect levels of a single toxicant to predicted probable long-term adverse effects on fish from acute tests. The concept was proposed by Mount and Stephan[27] as a way to determine safe levels of toxicants for species which could not be tested on a long-term basis. Initially many workers in the field hoped that a common, if not exact, factor might be proposed which would permit extension to many toxicants and species. Their work did not support this assumption. Failing this result, a more restricted objective was sought, namely, use of a single factor for a single toxicant that would be applicable across a wide spectrum of species. The National Academy of Science Panel on Water Quality Standards[28] accepted this general approach and assigned an application factor for use with all toxicants on which acute toxicity data but not chronic toxicity data were available. In general, the most sensitive species was used as a basis for final recommendation.

Subsequent research has shown that a single factor cannot be used for all toxicants and that with a single toxicant, its application to many species may not be tenable.

In the study reported here several species of fish and invertebrates were tested under the same conditions in flow-through apparatus under one principal investigator and, for the most part, by the same staff. In the seven species of fish tested, LTC values were used where possible or 96-hr LC50 where threshold values were not available (Tables 134, 135). Chronic test temperatures and acute test temperatures as close as possible were used for comparison. Estimates of no-effect levels were based on survival, growth, or success of reproduction, whichever showed the greatest sensitivity. Application factors were calculated by dividing no-effect concentrations established for the most sensitive criterion by the acute toxic value for various life history stages. The no-effect values may not be precise since the true no-effect level is usually between the observed no-effect test concentration and the lowest concentration showing adverse affects. Various methods have been proposed for surmounting this difficulty. In the present report the highest test concentration which showed no effect has been used as the "no-effect" level.

Calculation of a meaningful application factor is complicated by several factors. The first is related to the life history stage used for the acute base level and whether a 96-hr or LTC test is employed. A second is the criterion of no-effect concentration. This criterion may be growth, long-term survival, normal reproduction when entire life cycle from egg to egg is covered, or various performance and behavioral observations. Meaningful assessment is further complicated by relating observed chronic responses to their true ecological significance. A third limitation is the selection of appropriate test temperatures for establishing the base values. As data on H_2S show, response may vary drastically with changes in temperature. Tests on which application factors are based in this study were done at varied temperatures but are those which in general would be found in nature during the corresponding stage of life.

Table 134. NO-EFFECT CONCENTRATIONS OF H_2S FOR VARIOUS STAGES AND LTC CONCENTRATIONS FOR JUVENILES IN SEVEN SPECIES OF FISH

(mg/liter H_2S)

	Original values					Corrected values				
Species	Spawning	Juvenile growth	Fry survival	Hatch success	Juvenile LTC (days)	Spawning	Juvenile growth	Fry survival	Hatch success	Juvenile LTC (days)
Brook trout	.0055	.0067	-	-	.0155(6-11)	.0079	.0092	-	-	.0223(6-11)
Rainbow trout	-	.0059	-	.0025	.0087 (17)	-	.0085	-	.0035	.0121 (17)
Northern pike	-	-	.004[a]	.014[a]	-	-	-	.006[a]	.020[a]	-
Fathead[c] minnow	.0035	.0033	.0035	.0035	.0120(8-10)	.0052	.0049	.0061	.0061	.0182(8-10)
Goldfish	.0050	.0070	.0207	.0097	.0710 (11)	.0070	.0101	.0306	.0142	.1027 (11)
Walleye	-	-	.007[b]	.012[b]	.0193 (4)	-	-	.010[b]	.017[b]	.0282 (4)
Bluegill	.0007	.0015	-	-	.0318(8-10)	.0010	.0022	-	-	.0481(8-10)

[a] Adelman and Smith.[2]

[b] Smith and Oseid.[18]

[c] Duluth stock.

Table 135. LC50 (96-HOUR) AND LTC VALUES OF JUVENILES AND APPLICATION FACTORS BASED ON CHRONIC NO-EFFECT LEVEL[a]

Species	Juvenile		Chronic no-effect level, mg/l H_2S	Application Factor	
	96-hr LC50, mg/l H_2S	LTC, mg/l H_2S		96-hr LC50	LTC
Brook trout[b]	0.0266	0.0155	0.0055	0.207	0.355
Rainbow trout[b]	0.0130	0.0087	0.0025	0.192	0.287
Fathead minnow (D)[c]	0.0162	0.0120	0.0033	0.203	0.275
Fathead minnow (F)[d]	0.0367	-	0.0041	0.112	-
Goldfish[b]	0.0830	0.0710	0.0050	0.060	0.070
Walleye[b]	0.0193	-	0.0031	0.161	-
Bluegill[b]	0.0316	0.0318	0.0004[e]	0.013	0.013
Gammarus	0.0220	0.0110	0.0020	0.091	0.182
Hexagenia[b]	0.1650	0.0600	0.0150	0.091	0.250
Procambarus	0.0830	0.0530	0.0040	0.048	0.076

[a] These comparisons made on basis of values uncorrected for new ionization constants and criterion showing lowest no-effect level; acute values based on temperature most similar to mean chronic temperatures.

[b] Fish subjected to partial chronic tests and not through entire life cycle (egg-to-egg).

[c] Duluth stock.

[d] Field stock.

[e] Based on swimming performance.

Most acute testing is based on response of juvenile fish and usually for a 96-hr period or, in some cases, on LTC. Long-term chronic tests usually do not include an egg-to-egg cycle, the most desirable no-effect base.

As pointed out above, the application factor will vary in a single species depending on the life history stage used for comparison. When juveniles at a selected temperature (trout, 15 C and bluegill, 20 C) are compared to the most sensitive stage, the factor will be about 3-4 times higher if figures for the most sensitive stage are used. When application factors for various species based on acute tests with juveniles are calculated at temperatures presumed to be preferred by this stage, a large difference is found (0.231 for rainbow trout, 0.013 for bluegill).

Depending on the criterion used for no-effect, application factors may vary considerably. In the case of bluegills, if growth of juveniles is used to determine the no-effect level, the factor will be 0.13, but if the reproductive success of adults is compared to acute response of juveniles, the factor will be 0.013. Even this factor may not give true protection since at the lowest concentration tested (0.0007 mg/liter H_2S) some inhibition of egg deposition occurred.

Another factor complicating assessment of appropriate application factors is the temperature at which acute tests are made. As was demonstrated in fathead minnows and goldfish, the differences may be extreme. When application factors were calculated for the various temperatures, a wide range of results was obtained (Table 136). In the fathead minnows, the difference in application factors may be twentyfold (0.005 to 0.100) depending on the temperature of acute tests. With goldfish it can be fifteenfold. Tests at the same temperatures were not conducted on all species. It was, therefore, not demonstrated that the relative difference in application factors between species would be the same at all temperatures. Application

Table 136. COMPARISON OF APPLICATION FACTOR FOR H_2S WITH FATHEAD MINNOWS AND GOLDFISH TESTED FOR ACUTE RESPONSE AT DIFFERENT TEMPERATURES[a]

Fathead minnow			Goldfish		
Temperature, C	96-hr LC50 mg/1 H_2S	Application factor	Temperature, C	96-hr LC50, mg/1 H_2S	Application factor
6.5	0.580	.005	6.7	0.556	.009
7.6	0.520	.006	10.2	0.271	.019
10.0	0.150	.020	12.4	0.175	.029
15.0	0.057	.050	17.0	0.053	.094
20.3	0.032[b]	.930	20.3	0.048	.104
25.0	0.028	.100	24.8	0.037	.135

[a] No-effect level for fathead minnow 0.003 mg/liter and for goldfish, 0.005 mg/liter.

[b] Single test in temperature series (Table 4).

factors applied to invertebrates are within the range exhibited by fish species.

In view of the foregoing considerations, it is apparent that determination of safe levels or no-effect levels should be based on a test of the full life cycle. Reproduction may not be more sensitive than other responses with some toxicants and species; but, until this is demonstrated, assessment of safe levels for growth, survival, or physical performance must remain an approximation. In the setting of water quality standards and appraisal of effect on ecosystems a "no-effect" level may not be required to maintain an acceptable or even normal fish population structure and production.

REGIONAL DIFFERENCE IN FISH TOLERANCE

Acute tests on fathead minnows from different populations and limnological sites showed that the nature of the habitat made little difference but that populations from widely separated areas, such as Minnesota and southern Ohio, may have significantly different responses to H_2S. This fact makes the use of application factors based on acute tests from one population and chronic tests from the same species from a widely separated population of doubtful value.

NEW IONIZATION CONSTANTS

In Part II of this report (published separately) an analytical technique for molecular H_2S is described, and a new calculation of ionization constants has been prepared. A factor of 1.35-1.52 must be applied to the Pomeroy constants[7] to obtain revised values in the range of pH 7.5-8.0 and temperatures of 10-25 C. The summary tables in the discussion section show corrected values taken from the complete table as well as those originally calculated from the Pomeroy tables and reported in the body of the report. It is believed that the new constants will not affect the basic recommendations which have been made for standard-setting purposes.

SECTION XVI

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SECTION XVIII

GLOSSARY

Acute Test - Bioassay with duration less than 3 weeks with effect measured by survival.

Acute Toxicity - Lethal toxicity occurring within 12 hr to 3 weeks.

Adults - Fish which have spawned or are capable of spawning during the current reproductive season.

Application Factor - The quotient of the no-effect concentration of a toxicant for a fish population divided by the LC50 concentration for the same toxicant and population: no-effect concentration/ 96-hr LC50 concentration or LTC.

Chemical Metering Apparatus (CMA) - Sometimes referred to as "dipping bird" apparatus - a dispensing system (Mount and Warner 1967) used to inject toxicants to test chambers on a cyclic basis determined by water discharge.

Chronic Test - A bioassay which exceeds 3 weeks with effects measured by growth, fecundity, behavior, or survival.

Chronic Toxicity - Adverse effect on growth, fecundity, behavior, or survival in tests exceeding 3 weeks in length.

Crayfish, adult - Organisms which have spawned or exhibit reproductive behavior during the normal spawning period.

Crayfish, berried female - Female with eggs attached to swimmerettes.

Crayfish, juvenile - Crayfish from 3rd instar to development of reproductive capacity.

Crayfish, larvae - Newly hatched crayfish through the first two instars.

Crayfish, subadult - Organisms which will reproduce during the next natural spawning season.

Eggs, eyed - Eggs in which embryos have eyes well pigmented and distinguishable without magnification and which will hatch in several days under normal conditions.

Eggs, green - Eggs which have been fertilized but have not gone beyond the primary cleavages.

Fingerlings - Fish after absorption of the yolk sac and assumption of normal feeding patterns, usually reserved for fish through the first season of growth.

Fry - Fish which have not yet reached fingerling stage and which may have yolk sac still unabsorbed.

Juveniles - Usually synonymous with fingerlings but may be applied to fish in the second season of growth.

LC50 - The concentration of a toxicant which will cause 50% of test organisms to be dead at a specified time, commonly 24, 48, 96 hrs up to 3 weeks.

Lethal - Used with reference to toxicant concentrations which cause death within short periods.

Lethal Threshold Concentration (LTC) - The calculated LC50 concentration obtained in an acute test at a point in time when no change in LC50 has occurred for 48 hr (sometimes designated as asymptotic LC50).

Molecular H_2S - That portion of dissolved sulfide ($H_2S + HS^- + S^=$) which is unionized and does not include HS^- or $S^=$.

No-effect Concentration (Safe Level) - The concentration of a toxicant which will permit organisms to complete various life history stages without apparent adverse effects.

Sac Fry - Any fish still carrying unabsorbed yolk sac.

Safe Level - See "No-effect Concentration."

Subadults - Juvenile fish capable of spawning during their next reproductive cycle but which have not yet shown reproductive behavior, coloration, or secondary sex characteristics.

Sublethal - Used with reference to toxicant concentrations which permit survival without reference to possible adverse effects on growth, reproduction, behavior, etc.

Swim-up Fry - Fry which have left the bottom and swim normally through test chamber and accept food but still retain some of yolk sac.

Young-of-the-year (yy) - Fish during first season of growth.